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BACTERIOLOGIC STUDIES OF THE BLOOD OF NORMAL INDIVIDUALS AND RHEUMATIC FEVER PATIENTS*

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THIS work began almost four years ago, soon after Kendall[1] described his new medium. The results reported in this paper deal only with the bacteriologic findings obtained by serial transfer technic. Each blood sample was divided into two parts; one portion was cultured on Kendall's fluid media (E. M.); the other portion of the same specimen was cultured on dextrose calcium carbonate veal infusion broth (V. R.). After incubation of these flasks, heavy inocula were transferred to surfaces of lactose agar plates and transferred in series as first described by Hauduroy[2] and modified by Ryan.[3] Bloods of normal children, rheumatic fever children, and normal adults were investigated by this method. There are many reports appearing in the current literature on the bacteriology of rheumatic fever. Various types of dissociated bacteria have been described. It is not possible with our present knowledge to evaluate the significance of these findings in rheumatic fever cases.

Clawson[4] and Callow[5] have reviewed the literature on the bacteriology of the blood in rheumatic fever patients.

Technic.—After applying tincture of iodine to the arm site and then removing it with 96 per cent alcohol, 10 c.c. of blood was drawn into a sterile syringe. The blood was immediately transferred, using aseptic technic, to a sterile test tube and placed in the ice chest. (All syringes were rolled in towels and autoclaved at 15 pounds' pressure for twenty-five to thirty minutes.) The needles were placed in cotton stoppered small tubes and heated in the hot air oven at

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190° C. for one hour. The syringes were assembled immediately preceding use. After remaining in the ice chest overnight, the serum was removed with a sterile pipette and the remaining clot was then divided as nearly as possible into two equal portions. One part of the serum and clot was in each instance added to 100 c.c. flasks of Kendall and veal infusion dextrose broth media. The Kendall's medium was made up according to the published record.[1] The dextrose carbonate broth was prepared as follows: Five hundred grams of lean veal, 20 gm. of Difco peptone, 5 gm. of sodium chloride, and 1,000 c.c. of distilled water; adjusted to pH 7.8; and calcium carbonate added to each flask (100 c.c.) and then autoclaved. Two cubic centimeters of a 20 per cent Berkefeld filtered dextrose was added to each flask before inoculation, with the clot. All flasks were incubated at 37° C.

A modification of Clawson's[4] technic was used. Gram-stained smears were prepared from each inoculated flask every forty-eight hours; for every two seeded flasks there was one uninoculated 100 c.c. flask control included in our series. In no instance have we found a growth after 20 serial transfers on our control sterile medium. As soon as organisms were detected in the stained films, 0.2 to 0.3 c.c. of the culture was seeded onto a sterile lactose agar plate. Incubation of all other flasks not showing bacterial forms on smears was continued for fourteen days and then the flasks were routinely seeded in the same manner to lactose agar plates. All plates were incubated at 37° C. and examined carefully every twenty-four hours with the low power lens of the microscope. If no growth appeared after forty-eight hours, serial transfers were started, using Ryan's[3] modification of Hauduroy's[2] technic. In the event that no growth occurred after twenty serial transfers on lactose agar, the series was then discontinued and recorded as negative. All lactose agar plates used in this work were incubated overnight and carefully examined for any minute colonies before being used. The rim of the dish was flamed in the Bunsen burner before and after being opened. Only the central portion of each dish was used in spreading the inoculum. Gram-stained films were prepared systematically as soon as growth was observed on the plate. All original flasks were allowed to remain in the incubator from four to six weeks and before being discarded, agar plates were spread, in order to determine whether growth had taken place in the flasks after prolonged incubation.

Space does not permit long detailed protocols, although we realize these would give most information. Previous workers have not reported their results in sufficient detail for us to compare with our observations. We are recording the first five cultures carried out in serial transfer to indicate our findings with each of the three series of bloods examined. We do not see any advantage of Kendall's medium over the dextrose carbonate veal infusion broth. Table IV gives a résumé of our findings. It will be noted that growth occurs using serial transfers after carbonate broth a little sooner than after Kendall's medium. This holds for normal children, rheumatic fever children and normal adults. The material was removed for Kendall's medium study first and then the remaining blood sample used for the dextrose carbonate broth, which most probably explains the higher incidence of contamination in the latter series of tests.

Typical cultures obtained by the serial transfer method from blood of two normal children, one normal young adult, and two rheumatic fever children were selected for some immunologic experiments. Lactose agar plates were seeded and growth washed off with saline. Rabbits were injected intravenously with fresh

TABLE I

RECORD OF BACTERIOLOGIC RESULTS OF BLOOD OF NORMAL ADULTS OBTAINED BY SERIAL TRANSFER METHOD. KENDALL AND CALCIUM CARBONATE MEDIA WERE BOTH USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	8	R	Diphtheroid	2+	
	$CaCO_3$	1	R	Diphtheroid	2+	Salmon pigment
2	K	7	R	Diplococci	2+	
	$CaCO_3$	4	R	Diplococci	4+	
3	K	--	--	----	--	Negative up to twentieth serial plate
	$CaCO_3$	--	--	----	--	Negative up to twentieth serial plate
4	K	8	R	Diplococci	2+	Short chains in broth
	$CaCO_3$	4	R	Diplococci	4+	
5	K	10	R	Minute diphtheroids	3+	Short chains and irregular groups in broth
	$CaCO_3$	4	R	Diphtheroids	3+	

TABLE II

BACTERIOLOGIC FINDINGS BY SERIAL TRANSFER METHOD OF BLOOD OF NORMAL CHILDREN. KENDALL'S MEDIUM AND CALCIUM CARBONATE BROTH WERE USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	6	R	Diphtheroids	2+	Orange pigment on ageing
	$CaCO_3$	1	R	Diphtheroids	1+	
2	K	1	R	Diphtheroids diplococci	2+	
	$CaCO_3$	4	R	Diphtheroids	1+	
3	K	5	R	Diphtheroids	2+	Rose pigment after several transfers
	$CaCO_3$	5	R	Diphtheroids	1+	
4	K	9	R	Slender diphtheroids	1+	
	$CaCO_3$	3	R	Diplococci diphtheroids	1+	
5	K	4	R	Gram + bloated nodular rods	1+	
	$CaCO_3$	5	R	Large diphtheroids	1+	

living suspensions twice weekly for five weeks; 1 and 2 c.c. of the suspensions were injected. Trial bleedings were made after the fifth injection. One week after the last injection animals were bled from the carotid artery. No detectable reaction occurred during course of injection in rabbits. No agglutinins were demonstrable in any antiserums tested at any time. There were no pathologic lesions in rabbits at autopsy after carotid bleeding. The bacterial suspensions were used as antigens with serums of the same subject; no agglutinins could be demonstrated.

We were unable to find significant differences between the bacterial flora of the bloods of normal and rheumatic fever children and normal adults using serial transfer technic. All of these specimens would be considered sterile by the usual diagnostic laboratory methods.

TABLE III

BACTERIOLOGIC STUDIES OF BLOOD OF RHEUMATIC FEVER CHILDREN BY SERIAL TRANSFER METHOD. KENDALL'S MEDIUM AND CALCIUM CARBONATE BROTH WERE USED FOR EACH SAMPLE

PATIENT	MEDIUM	SERIAL	COLONY TYPE	GRAM STAIN	BROTH GROWTH	COMMENTS
1	K	4	S	Diphtheroids	1+	Yellow pigment after two transfers on Lac. agar; then lost pigment
	$CaCO_3$	5	R	Diphtheroids	1+	
2	K	3	R	Diplococci diphtheroids	1+	
	$CaCO_3$	3	R	Diplococci diphtheroids	1+	
3	K	3	R	Gram-positive diplococci	3+	
	$CaCO_3$	3	S	Giant cocci	3+	
4	K	6	S	Diphtheroids	1+	
	$CaCO_3$	3	R	Diphtheroids	1+	
5	K	3	R	Diphtheroids	1+	
	$CaCO_3$	3	R	Diphtheroids	1+	

TABLE IV

RÉSUMÉ TABLE OF SERIAL TRANSFER RESULTS OBTAINED FROM BLOOD OF NORMAL CHILDREN AND ADULTS AND OF RHEUMATIC FEVER CHILDREN

CULTURES	CHILDREN				NORMAL ADULTS	
	NORMAL		RHEUMATIC			
	"K"	$CaCO_3$	"K"	$CaCO_3$	"K"	$CaCO_3$
Average Serial transfer on which growth appeared	8.6	6.0	6.1	5.0	7.5	5.2
"Sterile" Negative after twenty transfers	3	2	0	0	5	2
Contamination	2	3	3	6	4	9

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XYLOSE TOLERANCE OF RABBITS WITH URANIUM NEPHRITIS*

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THE clearances of ingested foreign sugars have assumed considerable importance with their use as kidney and liver function tests. This investigation is concerned with xylose tolerance of normal rabbits and of rabbits with uranium-induced nephritis. Since it has been claimed[1-3] that xylose clearance is a delicate index of renal function, it was presumed that the impairment of function caused by the injection of very small quantities of uranium acetate would be indicated by a slight lowering of the xylose tolerance. The results of the administration of these small doses are of interest from the standpoint of both xylose tolerance and uranium intoxication. A perusal of the voluminous literature on uranium poisoning has yielded little concerning the effect on the kidneys of very small quantities of this toxic substance, or the progressive effect and the degree of uranium resistance developed when repeated and increasing small doses are given.

EXPERIMENTAL

Rabbits varying in age from one and one-half to four years were used for the experiment. A preliminary xylose tolerance test performed on each animal indicated normal kidney function. The animals were given, without preliminary fasting, 1 gm. of xylose per kilo body weight by stomach tube, 50 per cent sugar solutions being administered and washed down with 25 c.c. H_2O. The nonfermentable sugar of the blood was then followed for five hours. The rabbits were bled before the sugar was given and at hourly intervals thereafter, the blood being obtained from the marginal vein of the ear. A 0.2 c.c. blood sample was laked in 3.8 c.c. H_2O in a centrifuge tube, 1 c.c. of a 25 per cent suspension of yeast (washed three times with H_2O) added, and the tubes allowed to stand one-half hour at room temperature. Five cubic centimeters of tungstic acid made from equal parts of H_2SO_4 (120 c.c. of 0.666 N H_2SO_4 in 1 liter) and sodium tungstate (120 c.c. of 10 per cent $Na_2WO_4.2H_2O$ in 1 liter) were then added, the tubes shaken and centrifuged. Five cubic centimeters of the tungstic acid filtrate were used for the nonfermentable sugar determination by the method of Folin and Malmros.[4] Urea nitrogen determinations on 0.2 c.c. blood were made simultaneously with the xylose tests using the manometric method of Van Slyke.[5] Uranium acetate in amounts varying from 0.2 mg. to 1 mg. per kilo was injected subcutaneously, and the xylose tolerance run two to four days after injection, or in some cases the sugar tolerance was determined daily after injection in order to ascertain the period of maximum effect. For purposes of

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studying the effect of liver function on the clearance of xylose, a few experiments were undertaken in which the rabbits were poisoned by subcutaneous injection of phosphorus in olive oil in amounts varying from 0.4 to 1 mg. per kilo. In the phosphorus experiments, after the administration of xylose, 0.2 gm. glycine per kilo was injected into the marginal vein of the ear, and the amino nitrogen[6] of the blood determined before and two and one-half hours after injection. At the conclusion of each experiment, the animal was killed and an autopsy was performed. Urine, removed from the bladder, was tested for albumin and the sediment was examined; the kidneys and livers were saved for histologic examination.

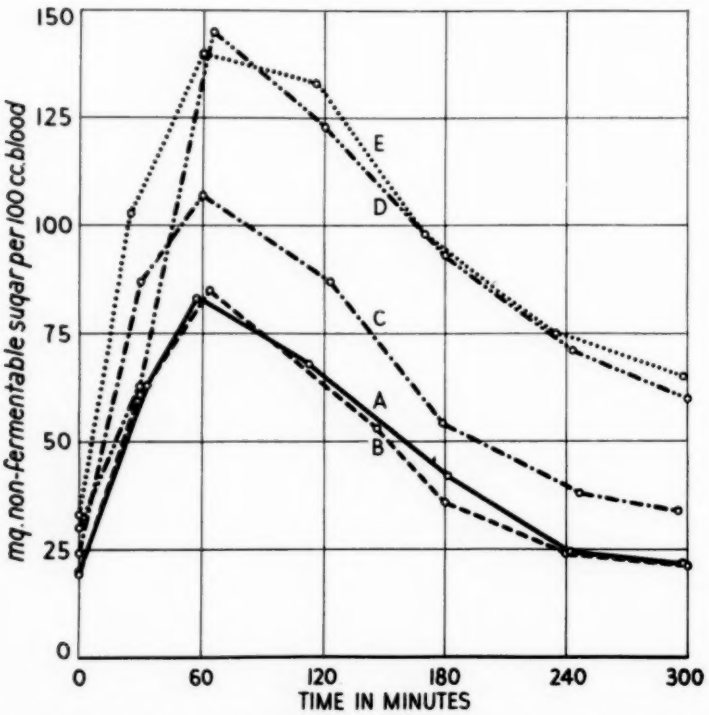

Fig. 1.—Effect of uranium intoxication in a rabbit as shown by the xylose tolerance. Normal tolerance curve *A*, before uranium injection; *B*, seventeen hours; *C*, two days; *D*, three days; *E*, four days after an injection of 0.5 mg. uranium acetate per kilo. Maximum effect occurs third or fourth day.

XYLOSE TOLERANCE

When xylose is administered orally to normal rabbits, the nonfermentable sugar of the blood rises to a maximum within one or two hours, after which it falls gradually until the initial or normal value is approached within five hours. According to Fishberg and Friedfeld[1-3] if the kidney function is impaired, the nonfermentable blood sugar may continue to rise even after five hours; and a correspondingly longer period of time, depending upon the amount of kidney damage, is required before the xylose is eliminated from the blood stream. It was thought that the course of uranium poisoning would manifest itself by progressively lower tolerance.

In Fig. 1 are shown the daily xylose tolerances of a rabbit after a single administration of 0.5 mg. uranium acetate per kilo. Seventeen hours after the uranium was given, the xylose curve checked with the normal. In this period the blood urea nitrogen had risen from 11.4 to 27.4 mg. The second day showed a considerable rise in the xylose curve, followed by a still greater rise on the third day. The fourth-day tolerance paralleled the third, so that the maximum effect of a single injection of uranium is reached about the third day after administration. This maximum effect varies in different animals, but appears between two and four days after injection.

In the present work, an effort was made to give the smallest dose of uranium which would produce sufficient kidney damage to be measured by the xylose

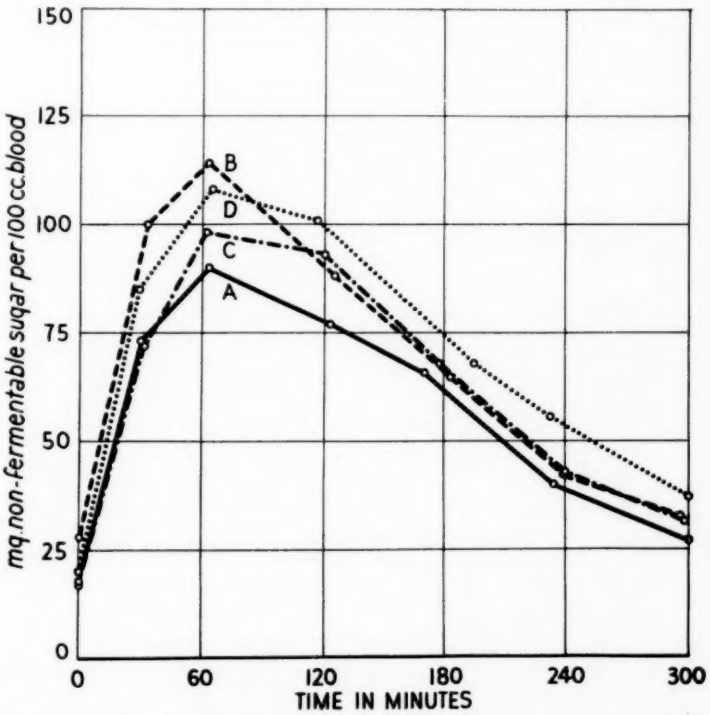

Fig. 2.—Acquired uranium resistance in a rabbit which had previously been intoxicated by small amounts of uranium. Xylose tolerance *A*, two months after 0.5, 0.5 and 1.0 mg. per kilo doses of uranium acetate; *B*, tolerance three days after additional 0.5 mg.; *C*, three days after 0.6 mg.; *D*, three days after 0.7 mg. uranium acetate per kilo, 11 days intervening between doses.

clearance, and then to increase this dose gradually. The susceptibility of rabbits to uranium varied greatly; some reacted to such small amounts as 0.2 or 0.3 mg. per kilo while with others these quantities seemed to have no effect. Five-tenths milligrams per kilo was found sufficient to produce the initial effect in most rabbits, but in three cases was enough to cause death. The rate of absorption of uranium also seemed to vary in different rabbits, and must be considered in determining the minimum toxic dosage. Age and sex must also be considered.

After repeated small doses, rabbits seem to develop a resistance to uranium intoxication. This is contrary to the work of Garnier and Marek[7, 8] who claim

that rabbits become accustomed to uranium only after increasing doses from 1 to such amounts as 40, 60, and 80 mg. per kilo with suitable time intervals intervening. They claim[9] that small doses and repetition of the same dose do not impart resistance, but that nitrogen excretion increases. According to Patrassi and Rogers,[10] rabbits treated with repeated small subcutaneous doses of uranium develop kidney lesions of a lesser degree on subsequent intravenous treatment than do control animals which have received intravenous injections of uranium without previous subcutaneous treatment. MacNider[11] has recently shown that dogs may develop resistance to uranium with repetition of 4 mg. per kilo doses. He found that in animals which survived this injection there was evidence of great disturbance in hepatic function as shown by the use of the phenoltetra-

TABLE I

XYLOSE TOLERANCE OF RABBITS BEFORE AND THREE DAYS AFTER INITIAL URANIUM INJECTION

RABBIT	URANIUM ACETATE	NONFERMENTABLE BLOOD SUGAR MG. PER 100 C.C. BLOOD							BLOOD UREA N
		BEFORE XYLOSE	MINUTES* AFTER INGESTION OF XYLOSE						
			30	60	120	180	240	300	
	mg. per kilo	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg. per 100 c.c.
113 ♀	0	24	65	84	72	52	35	26	13.0
	0.5	27	84	92	84	61	40	34	17.1
170 ♀	0	25	37	79	66	38	24	24	17.8
	0.5	29	75	84	74	61	43	33	16.8
188 ♀	0	18	46	85	59	35	25	18	13.0
	0.5	21	73	91	75	43	29	23	13.5
196 ♂	0	24	60	78	52	42	35	26	13.4
	0.5	24	61	71	56	53	46	37	18.3
223 ♀	0	26	36	88	87	56	45	31	14.8
	0.5	26	80	123	136	124	83	39	19.5
297 ♀	0	24	64	84	80	63	40	27	14.9
	0.5	28	56	83	98	71	52	32	18.0
304 ♀	0	23	66	73	54	43	31	20	11.0
	0.5	26	72	75	62	37	30	25	22.0
347 ♀	0	19	63	76	66	44	31	21	16.3
	0.5	25	67	97	89	67	44	28	27.1

*Approximate.

chlorphthalein test. In animals surviving such a degree of liver injury there slowly occurs an improvement of hepatic function. Biopsy material obtained from the liver at this stage showed that the repair process to the epithelium had taken place through the formation of a different type of cell. When these animals were reintoxicated with uranium, it was found that the liver had acquired resistance to this hepatoxic substance. MacNider[12, 13] also found that the injection of 4 mg. per kilo uranium nitrate in dogs resulted in an epithelial injury to the kidney which was very largely localized to the proximal convoluted tubule epithelium. A certain number of dogs rendered acutely nephritic returned to complete normal function after a variable period allowed for renal repair. He found that restoration of such a function was accompanied by regeneration of epithelium of two different types. In one, the regenerated epithelium resembled histologically normal convoluted tubule cells while in the other, in

addition to these normal cells, there was a dominance of a low, flattened, apparently less specialized epithelium. Animals in which this latter type occurred were found to have developed a uranium resistance.

In this investigation relatively small amounts of uranium were used in comparison with those employed by MacNider, and Garnier and Marek. Uranium acetate in amounts from 0.2 to 1.0 mg. was employed. The usual dose was 0.5 mg., and the reactions of the rabbits to this amount varied greatly. The majority showed a considerable decrease in xylose tolerance after initial

TABLE II

XYLOSE TOLERANCE OF RABBITS AFTER REPEATED INJECTIONS OF URANIUM

DATE	RABBIT	NONFERMENTABLE BLOOD SUGAR MG. PER 100 C.C. BLOOD							BLOOD UREA N	URANIUM ACETATE INJECTED	
		BEFORE XYLOSE	MINUTES* AFTER INGESTION OF XYLOSE						MG. PER 100 C.C.	DATE	MG. PER K.
			30	60	120	180	240	300			
		mg.	mg.	mg.	mg.	mg.	mg.	mg.			
5/15	35 ♂	21	63	89	87	56	36	29	22.9	5/20	0.5
5/23		27	92	126	133	111	104	75	31.4		
6/14		24	68	86	90	54	37	28	16.2	6/15	0.5
6/20		22	86	107	92	58	36	26	26.5	6/23	0.3
6/27		24	92	113	93	69	44	33	17.0	7/ 8	0.5
7/11		27	92	110	103	72	57	43	47.1		
3/28	164 ♀	22	59	76	60	43	25	20	13.2	4/ 8	0.5
4/11		20	47	70	82	76	65	52	54.4	4/22	0.6
4/24		23	73	104	111	81	58	43	20.1	5/ 1	0.7
5/ 4		26	59	93	105	97	68	44	17.2	7/14	0.5
7/17		26	60	80	86	69	47	37	23.2		
5/19	188 ♀	18	46	85	59	35	25	18	13.1	5/26	0.5
5/29		21	73	91	75	43	29	23	13.5	6/11	0.5
6/16		21	77	89	53	43	23	20	12.7	6/22	0.3
6/26		22	51	102	94	53		35	16.6	7/10	0.5
7/13		29	99	81	71		56	37	12.6		
3/30	326 ♂	18	59	77	56	35	25	18	11.5	4/10	0.5
4/13		20	90	113	118	105	89	63	36.8	4/24	0.6
4/28		22	89	107	114	100	73	52	19.3	5/ 5	0.7
5/ 8		21	68	91	111	83	58	40	20.9	7/15	0.5
7/19		24	37	66	75	57	37	27	26.2		
4/ 6	98 ♀ †	18	73	90	77	66	40	27	16.4	4/11	0.5
4/14		28	100	114	88	64	42	32	20.0	4/22	0.6
4/25		17	72	98	93	68	43	30	19.1	5/ 6	0.7
5/ 9		20	85	108	101	68	56	37	27.0	7/15	0.5
7/18		30	82	109	82	68	48	33	22.3		

*Approximate.

†This rabbit had previously received 0.5 mg. uranium per kilo on 12/11; 0.25 mg. on 12/16; and 0.5 mg. on 12/23 and 2/25.

uranium intoxication, but in approximately a fourth of the animals this small amount either had no effect or there was a barely perceptible decrease in tolerance.

In Table I are listed the xylose tolerances of rabbits before injection of uranium, and three days after the administration of a 0.5 mg. per kilo dose. Most of these rabbits show the minimum effect of this small dose. There is only a slightly lower tolerance as compared with the normal, and this lower tolerance is accompanied by a more pronounced elevation in blood urea nitrogen. Albumin varies greatly. In nephritic rats previously studied,[14] xylose tolerance and urea retention did not show such close agreement.

Rabbits which showed a high degree of initial uranium intoxication were used in the experiments to determine whether or not a resistance could be developed to this toxic substance. In Table II are listed a few examples of the xylose tolerances of rabbits after repeated injections of uranium. These rabbits show marked kidney damage as a result of initial uranium poisoning. The tolerance is considerably lowered after the injection of a small amount of uranium, and there is a pronounced rise in the blood urea nitrogen. When the dose is repeated or slightly increased, after a suitable time interval has elapsed, the kidney seems to have acquired a resistance to uranium. The xylose tolerance again approaches the normal, and the blood urea nitrogen falls. Repeated doses of uranium appear to have little effect.

If, however, the time interval between doses is too short or too long this resistance is altered. It appears, therefore, that this acquired resistance is not a permanent one. Approximately a fourth of the animals treated failed to develop a definite uranium resistance. The lowered xylose tolerances of these rabbits may be due to individual variations of the animals and to a lack of a suitable time interval between uranium intoxications.

Rabbit 98 is an example of a group of rabbits which had been repeatedly intoxicated with small amounts of uranium over periods of one to four months. The xylose tolerances were then determined, and the animals were given increased amounts of uranium with little or no effect upon the xylose tolerance or blood urea nitrogen. In a few instances the tolerances and blood ureas rose considerably but later returned to normal after additional uranium treatment. There are individual variations and exceptions in the work with some rabbits which are difficult to explain.

When the rabbits in the above experiments were killed they showed pathologic evidence of nephritis and marked liver involvement. Histologic examination exhibited severe renal lesions, and in some rabbits, evidences of spontaneous nephritis. This brings up the question as to whether xylose clearance is a satisfactory test of renal function. If the above tolerances are considered in the light of the histologic reports, the answer would be in the negative. It would seem that in animals evidencing such marked nephritis the amount of xylose retained in the blood should have been considerably higher than that which was found. Fishberg and Friedfeld,[1, 3] working with rabbits with uranium-induced nephritis, report increased nonfermentable blood sugar values five hours after the administration of xylose. In the present investigation out of some 80 tolerance tests, the maximum nonfermentable blood sugar was obtained within two hours after ingestion of xylose, and at the end of five hours most values approached the normal. It is, therefore, apparent that the marked degree of nephritis reported here was not enough to cause any great retention of xylose, and it is evident that xylose tolerance cannot be considered a delicate indicator of renal function.

XYLOSE TOLERANCE IN PHOSPHORUS POISONING

In the clearance of various foreign substances from the blood stream, there is always the question as to whether or not more than one organ is involved. Fishberg and Friedfeld[1-3] claim that liver injury does not affect the clearance

TABLE III

XYLOSE TOLERANCE OF RABBITS POISONED BY PHOSPHORUS

DATE	RABBIT	PHOSPHORUS MG. PER K.		NONFERMENTABLE BLOOD SUGAR MG. PER 100 C.C. BLOOD							BLOOD UREA N	GLYCINE INJECTION BLOOD AMINO ACID N	
		MG.	DATE	BEFORE XYLOSE	MINUTES* AFTER INGESTION OF XYLOSE							BEFORE	AFTER 2½ HOURS
					30	60	120	180	240	300			
				mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
2/15	9 ♂ †	0.2	2/15	28	74	78	58	39	32	29	25.8		
2/19		0.6	2/19	25	65	82	56	40	32	28	23.4	8.8	11.2
2/23				22	85	88	59	43	28	25	22.5	11.3	11.5
2/26	187 ♂ ‡	0.8	2/24	34	35	36	75	88	85	70	33.2	10.1	20.3
1/15	196 ♂	0.6	2/16	27	66	82	58	42	33	25	20.3		
2/20		0.6	2/19	28	42	65	99	107	103	99	29.1	9.2	23.0
3/ 3	385 ♀	1.0	2/26	31	43	58	66	71	80	71	23.4	12.2	20.0
3/ 2	394 ♀	1.0	3/ 1	25	44	71	55	48	33	27	20.8	9.3	10.5

*Approximate.
†One pole of left kidney ligated 12/4.
‡One pole of left kndney ligated 12/4. Right nephrectomy 12/26.

of xylose. In order to investigate this question rabbits were injected with various amounts of phosphorus in olive oil, and a few days later xylose tolerance and deaminizing experiments were run simultaneously. After the ingestion of xylose, 0.2 gm. glycine per kilo was injected into the marginal vein of the ear. The amino nitrogen of the blood was determined before injection, and after two and one-half hours. The results are given in Table III.

Some of the rabbits were strangely resistant to phosphorus poisoning. This may have been due to faulty absorption of the phosphorus. In these animals, destruction of the liver was not sufficient to interfere with its deaminizing function, and the xylose tolerance remained normal. However, in those animals in which there was any considerable rise in amino acid nitrogen of the blood, xylose retention was great. In three instances the maximum nonfermentable blood sugar maintained a practically constant high level for five hours after xylose administration. It would therefore seem that where liver injury is sufficient to affect its deaminizing function, this injury may be manifested by giving very high xylose tolerances. A right nephrectomy and a ligation of one pole of the left kidney had previously been performed on one of the animals (Rabbit 187) used in this experiment. In a control animal similarly treated, the xylose tolerance was normal.

GALACTOSE TOLERANCE

A considerable amount of work was done with galactose tolerance, but space limitation prevents a detailed report and the inclusion of tables. It was found that galactose tolerance in normal rabbits was practically the same as xylose tolerance. What was said about dosage and resistance to uranium poisoning in the preceding work with xylose is also true of galactose. The majority of animals responded to relatively small amounts of uranium as evidenced by a decreased galactose tolerance and an increase in the blood urea nitrogen. The small amounts of uranium given were probably not sufficient to cause liver injury and one may conclude, therefore, that the kidney injury by uranium poisoning caused retention of the galactose. This would seem to indicate that retention of galactose is not solely an indication of damaged liver function.

SUMMARY

After injection of very small amounts of uranium, xylose tolerance is decreased. After repeated injection of these small amounts, rabbits seem to develop a uranium resistance. Considering the extent of kidney damage produced by uranium, the tolerance of xylose cannot be considered a delicate indicator of renal function. When the liver is severely injured by phosphorus poisoning, xylose tolerance may be greatly decreased.

Galactose tolerance in normal rabbits is practically the same as xylose tolerance. Kidney damage produced by uranium poisoning may lower galactose tolerance.

The author is indebted to Dr. E. M. Medlar for the histologic examinations. He wishes to express his appreciation to Dr. N. R. Blatherwick for his many helpful suggestions, and to Phoebe Bradshaw, Anna Post, and Owen Cullimore for valuable assistance in the work. Pure xylose was generously furnished by the United States Bureau of Standards.

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AGRANULOCYTOSIS IN THE NEGRO*

Case Report With Etiology and Comment

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AGRANULOCYTOSIS is a rare disease in negroes. In 1930 Tally and Griffith[1] reported one other such case in which the neutropenia occurred following the administration of arsphenamine. Grady Hospital has 225 beds for negro patients, and in eight years only one individual with agranulocytosis has been admitted to its wards. In no instance has the disease been observed in the outpatient clinic where more than 20,000 negroes are treated each year. The occurrence of this malady so rarely in negroes necessarily calls for etiologic speculation, and it is important and of great interest to know, if possible, the reason for its rarity in the negro race.

The etiology of agranulocytosis has not been entirely disclosed. Kracke and Parker[2] have presented clinical and experimental evidence that benzamine drugs (chiefly amidopyrine) are the predisposing etiologic agents; and their observations and claims have been confirmed by other observers in America and European countries.

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As regards the infrequency of the disease, Kracke[3] feels that the negroes do not have neutropenia, because they do not use drugs such as amidopyrine. There seems to be sound reason for this opinion. We who are southern physicians are well aware of the negro's aptitude and ability to undergo severe pain. The negro may be extremely emotional at times, but he wears his pain and suffering with grace and humility. His therapeutic agents are limited by necessity to simple remedies such as liniments, castor oil, epsom salts, hot pepper, turpentine, roots and herbs. When a headache occurs he applies liniment, uses a charm, and wears off the pain; or he may apply a tight bandage about his skull to "squeeze out the misery." Not infrequently we observe negroes in our hospital who undoubtedly have angina pectoris, yet it is difficult to obtain a description of the anginal manifestations as we have them described by white people. The negro will simply refer to "misery in my chest" and let it go at that. We have long since learned that such complaints in negroes may mean many things. Negro women, in contrast with white women, seldom complain of menstrual pain; however, this particular problem would open a wide field for consideration which is unnecessary in this report.

The statements herein so far made are intended to indicate that inasmuch as the negro bears his pain more stoically than many of the white people, he therefore finds it unnecessary to buy "pain killers." It is not amiss also to refer to the economic side of the matter. Certainly the poorer classes cannot, or do not, buy expensive analgesics, and we also recall how few cases of agranulocytosis we have seen in the poorer white people. Those of this class who are suburban and who come to the white wards of Grady Hospital likewise seldom have agranulocytosis.

If the negroes do not have granulopenia because they do not use benzamine drugs, then Kracke's etiologic claim is herein strongly supported. If they have not agranulocytosis because of other reasons, then the field of etiologic investigation increases in interest and widens the research scope. It is in order, therefore, that all such patients be studied thoroughly and reports made until the question of etiology is solved.

CASE REPORT

T. F. (No. 72339.) Female, aged twenty-four years. Admitted to the hospital on June 3, 1935, and died June 13, 1935, following an illness of forty-five days' duration.

The chief complaints were of fever and headache, which had existed for five weeks prior to admission to the hospital. She had not been entirely confined to her bed until one week before admission, at which time she was given at the clinic an injection of milk, the resulting reaction forcing her to bed. A day later she developed a sore throat, a nasal discharge, and slight deafness. The throat soreness became severe and she could not drink fluids. She had pain in her face and neck, and about both sides of her cheeks.

Past History.—The patient had always suffered pain during menstruation, and in recent months the pains were quite severe. She has also had a vaginal discharge for some time past which had recently increased. She had no children nor had she any miscarriages. No other illness of importance was described except a mild chronic gastric distress, bleeding hemorrhoids, and constipation. She had lost ten pounds in weight in the last thirty days.

Physical Findings.—She was a well-developed and well-nourished female, whose blood pressure was 112 systolic and 74 diastolic. She had fever of 104° F. and a pulse rate of 100. Her pupils were equal. The sclera were slight yellow. Her neck was not rigid. There were dental caries and swollen inflamed gums. A diffuse pharyngeal inflammation of a brownish

red color, and a yellow postnasal discharge were present. The tonsils were apparently uninvolved. All other findings were normal except lower abdominal tenderness on pressure with a slight rigidity of the abdominal muscles, enlarged liver, cervical discharge, internal and external hemorrhoids, and probable tubal ovarian masses as indicated in pelvic examination.

Course in Hospital.—The patient lay quietly in bed. She became rapidly dehydrated. When moved about she complained of pain and weakness. Her throat lesions continued. A definite jaundice became marked. She remained conscious, but appeared to be quite seriously ill. Her fever continued and averaged 103.5° F. On the sixth, seventh, ninth, and tenth days she had chills.

On June 4, her first blood study revealed 3,100 leucocytes, 3,200,000 red cells, hemoglobin 60 per cent; of the leucocytes 64 per cent were lymphocytes. Twenty-four hours later there were 1,150 leucocytes per c. mm. with 72 per cent lymphocytes. On June 6, there were only 850 leucocytes per c. mm. of blood and only lymphocytes could be found. Later, on the same date, 400 white cells were present and on the twelfth day of June the total white blood count was 150 cells per c. mm. No granulocytes were found. During the interim the red cells had decreased to 2,100,000. The icterus index was 32 and the fragility test normal. Wassermann negative; Widal negative; nonprotein nitrogen 33 mg.; malarial parasites not found; coagulation time four minutes; bleeding time 1.5 minutes; platelets 128,000 per c. mm. of blood. Four blood cultures remained free of bacteria. On June 13, 1935, she became extremely weak and died.

Treatment.—The patient was given liver extract on June 11, 1935, though it was realized that little good could be accomplished. She was also given a transfusion, which was of no avail, and fluids, glucose, tepid sponges, douches, sodium perborate gargles and morphia. Unfortunately, when first admitted, she was given pyramidon, 10 gr. (only two doses). This drug was omitted two days later on account of the low leucocyte count. On the tenth day, sterile turpentine was administered, intramuscularly, but all treatment failed to produce beneficial response.

Discussion of Case.—This patient presented many of the cardinal features of primary granulopenia: weakness, sore throat, pain over face and absence of circulating neutrophiles with practically total absence of all cells at death. After the diagnosis was established, the etiologic agent was sought. The patient denied taking pyramidon or amidopyrine previous to her illness, nor had she taken any drug of an obscure type. The pelvic inflammatory disease did not impress her physicians as being seriously significant. There was a story related by her that does seem significant and that relates to her deafness, which had recently increased in severity. She admitted taking quinine to control her fever at the beginning of her illness, and quinine is a drug containing the benzamine nucleus. I am inclined to feel that she must have consumed considerable quinine to have caused progressive diminished hearing. With these facts at hand this patient may be considered a person with granulopenia probably resulting from quinine intoxication, possibly allergic, until further investigation discloses other etiologic agents. However, the possible connection is obscured by the administration of 10 gr. of pyramidon on admission, since it has been shown that this amount of this drug is capable of producing profound depression of the leucocyte count.[4-6]

Only one instance of agranulocytosis resulting from quinine has been reported.[7] In this regard it is hoped that someone will undertake to evaluate quinine as a possible cause of the disease. As a rule patients with malaria have leucopenic blood, and occasionally malaria, a curable preventable disease, causes death. I recall that in fatal cases, presumably nonrefractory to therapeutic quinine, those patients have marked leucopenia, at times terminal hemor-

rhages, and a mental confusion such as occurs often in the typical agranulocytic patient. The question arises, therefore, concerning the dangerous effects of quinine on the white blood cells of those patients, and the matter should be investigated, for it has long been known that quinine may produce allergic reactions.

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THE RATE OF DISINTEGRATION OF PLATELETS*

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THUS far studies on blood platelets have been limited largely to numerical determinations, at times supplemented by an examination of their morphologic features as revealed by dry, stained blood films. General observations on the disintegration of platelets have been made by a number of investigators, but the actual rate at which this disintegration proceeds has until recently received little attention despite the important rôle the fragility of platelets is considered to play in conditions such as hemophilia and hereditary hemorrhagic thromboasthenia. In this investigation simultaneous observations were made on the rate of disintegration, total and differential counts of the thrombocytes, and an attempt was made to analyze the results from the standpoint of their clinical significance.

A satisfactory method for determining the rate of disintegration of platelets should include simultaneous observations on the total and differential counts, thus correlating the qualitative properties, quantitative aspects, and morphologic features of the thrombocytes. The various methods that have been employed for studying the fragility of platelets can be generally divided into indirect and direct.

INDIRECT METHOD

In this method, advocated by Irish,[1] a suspension of platelets in hypotonic salt solution is brought in contact with platelet-free plasma or a solution of fibrinogen, thus completing the clotting system. The fragility is expressed as

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the concentration of sodium chloride just sufficiently hypotonic to cause disintegration of enough platelets to facilitate rapid clotting. The platelets are first washed free from plasma and then suspended in physiologic saline, a procedure involving unavoidable loss and destruction of some of the thrombocytes. The method, moreover, does not permit simultaneous total and differential platelet counts.

DIRECT METHODS

These methods involve the use of the microscope. The disintegration of platelets can be observed either by following the changes taking place in the thrombocytes found in one or more microscopic fields or by determining at regular time intervals their quantitative variations. The various platelet suspension media that have been employed for this purpose are: (1) fresh undiluted blood, (2) physiologic solutions, (3) anticoagulants, and (4) anticoagulant preservatives.

1. *Fresh Undiluted Blood.*—The earliest observations on the disintegration of platelets were made by employing platelets suspended in fresh undiluted blood. Later Mayer[2] and Stübel[3] and more recently Tait and Burke[4] and Ferguson[5] supplemented these studies by the use of the dark-field microscope. In undiluted blood, however, the thrombocytes break up so rapidly that it is impossible to correlate by accurate simultaneous observations the fragility with the total and differential counts.

2. *Physiologic Solutions.*—Platelets suspended in diluted blood disintegrate more slowly than when suspended in undiluted blood. Attempts have, therefore, been made to carry out in preparations of diluted blood simultaneous observations on the fragility and total differential counts of the platelets. Flössner,[6] Hofmann,[7] Boshamer[8] and Horwitz[9-11] employed Tyrode's[12] solution as diluent. This diluent is a good artificial nourishing fluid, but a poor platelet preservative. In this solution the platelets begin to disintegrate after about twenty to thirty minutes, a time interval too brief for accurate determination of the total and differential platelet counts. Birch[13] and Kugelmass[14] used hypotonic salt solution as diluent, making no attempt to correlate the fragility of the platelets with the total and differential counts. I have found both Tyrode's solution and physiologic saline extremely unsatisfactory. The formed cellular blood elements are poorly preserved in these diluents and become considerably distorted after a short time. The results obtained by employing these solutions appear to be entirely untrustworthy.

3. *Anticoagulants.*—Aynaud,[15, 16] Baar and Székely,[17, 18] Baar[19] and Kiguchi and Mizuta[20] employed sodium citrate as anticoagulant. Aynaud used also sodium oxalate. These anticoagulants, however, do not preserve platelets and therefore possess no advantages over diluents such as physiologic sodium chloride or Tyrode's solution. Preiss[21] who used a mixture of Tyrode's solution and heparin failed to observe any significant changes in the total platelet count at the end of one hour; the differential formula, however, at the end of the same period of observation revealed a decrease in the smaller platelets and an increase in the larger forms.

4. *Anticoagulant Preservatives.*—The anticoagulants employed here possess the property of preserving platelets. Aynaud[15, 16, 22] studied the disintegration of platelets by using as diluents solutions of sodium arsenite, urea and sodium metaphosphate. More careful observations on the rate of disintegration of platelets were made by Baar and Székely[17, 18] and König.[23] The former investigators used a 10 per cent solution of urea originally recommended by Kristenson[24] for platelet enumeration, the latter a 14 per cent solution of magnesium sulphate. These diluents are definitely hypertonic and readily break up platelets. Urea, moreover, hemolyzes red cells. The hemolysis of red cells, by increasing the osmotic pressure, produces further alterations in the platelets. Neither the magnesium sulphate nor the urea solutions, therefore, are suitable diluents for making accurate observations on the fragility, total and differential counts of the platelets.

METHOD

The Diluent.—In this investigation a solution of sodium metaphosphate was employed for determining the rate of disintegration, total and differential counts of the platelets. It has the following composition:

	GM. OR C.C.
Sodium metaphosphate (Howe and French)	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

The procedures can be carried out simultaneously since this diluent, originally recommended by Pratt[25, 26] for platelet enumeration, preserves the thrombocytes for a sufficiently long period of time, at least two hours. In fact, in a 5 per cent solution of sodium metaphosphate the total number of thrombocytes has been observed by Pratt[27] to remain at times unchanged for days.

Some investigators advise against the use of anticoagulant preservatives as blood diluents for determining the fragility of platelets, since these at times produce considerable alterations in the thrombocytes. As a matter of fact some changes in the platelets take place immediately upon their extravascular escape due to a number of unavoidable factors: contact with tissue juices and disintegration products of cells; changes in temperature, osmotic relations and hydrogen ion concentration; disturbances of normal blood gas equilibria due to the escape of volatile substances. Even physiologic sodium chloride solution has been shown by Morawitz,[28] Schittenhelm and Bodong[29] and Abderhalden and Deetjen[30] to damage platelets. Extravascularly the platelets are always abnormal regardless of the medium in which they are suspended. The abnormality in the thrombocytes may reach a considerable degree in diluents like magnesium sulphate or urea previously discussed. Sodium metaphosphate, however, as an anticoagulant preservative, has been shown by Morawitz[28] to possess the unusual characteristic of preserving the platelets without producing any changes in their ferments or precursors of ferments, so that platelets suspended in this diluent retain their properties of coagulating blood. Furthermore, in the concentration employed here the sodium metaphosphate solution does not produce any distortion of the cellular blood elements which appear perfectly normal for many hours. Aynaud[22] and Bürker[31] made similar observations. The sodium

metaphosphate solution, therefore, as a platelet preservative is to be preferred to the previously described diluents, as it produces minimum alterations in the thrombocytes.

The Total Platelet Count.—The method employed for the enumeration of the platelets is described by me in detail elsewhere.[32] It may be outlined briefly as follows:

> The palmar surface of the finger tip is punctured with an automatic lancet after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether. The first drop or two of blood is discarded. A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin, and the hand quickly turned over so that the palmar surface is directed downward. After a sufficiently large drop of blood has escaped into the drop of diluent, the mixture is applied to the surface of a small quantity (three to four drops) of diluting fluid contained in a paraffin cup. The mixture in the cup is stirred and then transferred by means of a paraffin-coated applicator to a glass slide; usually three preparations can be obtained as the quantity of fluid in the cup yields three large drops. A cover slip is placed over each drop and after the preparations have been allowed to stand for ten to fifteen minutes, a relative thrombocyte-erythrocyte count is made, using the oil immersion lens. Sealing the edges of the preparations with liquid petrolatum will prevent the production of air currents. An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined. With this method the average number of platelets per cubic millimeter in normal adults is about 500,000.

The Differential Platelet Count.—A complete discussion of the method employed for determining the size of the platelets in wet preparations and the clinical significance of the deviations from the normal will be found elsewhere.[33] The size of the platelets is estimated in a manner similar to the determination of the average diameter of red cells. The procedure is carried out simultaneously with the enumeration of the thrombocytes. The platelets, as observed in wet preparations, can be divided according to size into four groups: Group I, consisting of platelets whose size is one-quarter the diameter of a red cell, or about 1.8 microns; Group II, consisting of platelets one-third the diameter of a red cell, or about 2.5 microns; Group III, consisting of platelets one-half the diameter of a red cell or greater, or about 3.6 microns; and Group IV, consisting of irregularly-shaped platelets. The differential formulas from 45 normal adults yielded the following averages: Group I, 18.6 per cent; Group II, 63.3 per cent; Group III, 17.4 per cent; Group IV, 0.7 per cent. There is considerable evidence indicating that the smaller platelets, those belonging to Group I, are the younger forms.[33]

The Rate of Disintegration of Platelets.—The rate of disintegration of platelets in the sodium metaphosphate solution can be studied by mixing a drop of freshly drawn blood with several drops of diluent contained in a paraffin cup and then determining the variations in the platelet count at regular time intervals. The results obtained by using the blood from a normal young adult are shown in Chart 1. It is apparent from this chart that, during the first two hours, the variations in the number of platelets are slight. This is followed during the succeeding five to six hours by a considerable drop in their numbers. After this point the numerical variations are of small magnitude, owing to the

slow disintegration of the remaining platelets. Based on these observations a method was devised for determining the rate of disintegration of thrombocytes. The technic is as follows:

Capillary blood is mixed with the preserving fluid by the method previously described for enumerating platelets. Two separate paraffin cups, each containing several drops of diluted blood, are prepared. Initial total and differential platelet counts are obtained by using the solution contained in one of the cups. The remaining cup is placed in an ice box and kept there at a temperature of approximately 10° C. At the end of about eight hours, total and differential platelet counts are obtained by using the solution contained in the second cup.

The rate of disintegration of the platelets as revealed by the decrease in the total platelet count at the end of eight hours shows considerable variations in normal individuals. If the total number of platelets lost at the end of the

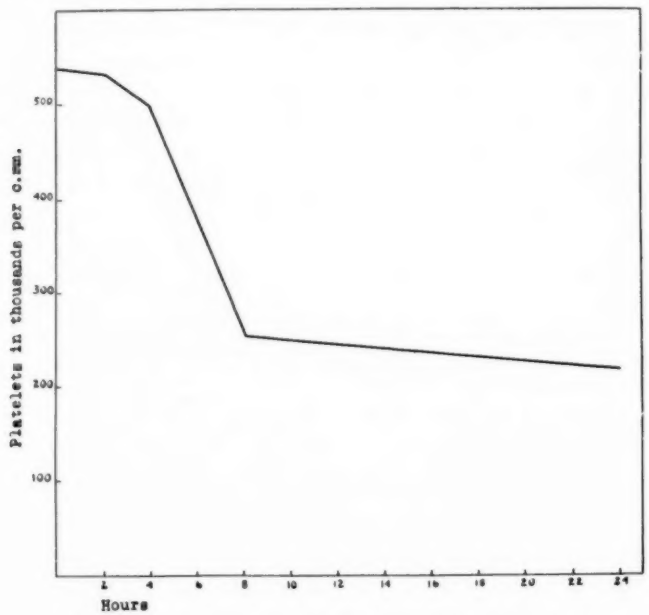

Chart 1.—Normal rate of disintegration of platelets.

observation period is divided by the initial count and the quotient multiplied by 100, the disintegration index (D. I.) in percentage is obtained. For example, if the initial count is 500,000 platelets per c.mm. and the eight-hour count 300,000, the disintegration index is 40; in other words, 40 per cent of the platelets have become completely disintegrated. In a group of 26 normal individuals 85 per cent revealed a disintegration index varying from 11 to 60, and 50 per cent an index ranging from 21 to 40.

The differential platelet formula undergoes very definite changes during the course of disintegration of the platelets. This is illustrated by Chart 2 and Table I.

Obviously the significant changes in the differential platelet formula at the end of eight hours are due to a relative and absolute decrease in the number of the larger platelets, i.e., those belonging to Groups II and III, and to a relative,

and usually also an absolute increase in the smaller forms, those belonging to Group I. The platelets of Group IV usually play a rather insignificant rôle. As a result of the disintegration of the platelets, there is a gradual shift of the differential formula to the left. The largest platelets, those belonging to Group III, presumably disintegrate into two or more fragments which then become members of either Groups I or II. The platelets of Group II disintegrate in turn to become units of Group I.

Apparently the larger, more mature platelets are more fragile and disintegrate more readily than the smaller platelets. Further support for this view is offered by the morphologic and quantitative changes of the thrombocytes following the subcutaneous injection of 1 c.c. of adrenalin chloride (1:1,000), as shown in Table II.

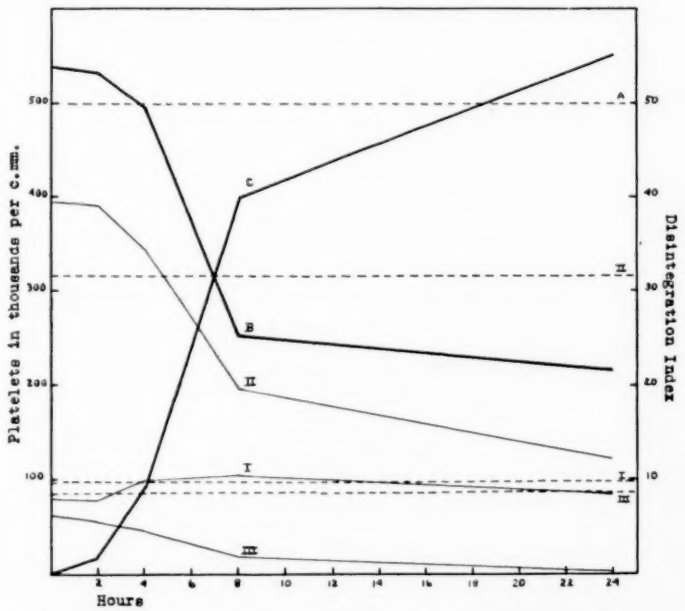

Chart 2.—Behavior of the disintegration index and the differential formula during the normal course of the disintegration of platelets. Dotted line *A* represents the normal platelet level, dotted lines *I*, *II*, and *III* the normal absolute levels of the corresponding groups. Continuous line *B* represents the behavior of the total platelet count of a normal subject, continuous lines *I*, *II*, and *III* the absolute counts of the corresponding groups. Continuous line *C* represents the behavior of the disintegration index.

The characteristic feature of adrenalin thrombocytosis is the appearance of increased numbers of large platelets which must come from inactive blood reservoirs where normally hematopoiesis does not occur. They must consequently represent platelets that have remained inactive in the blood depots where many of them have matured, and were forced into the general circulation following the injection of adrenalin; they are, therefore, older or senile platelets. Associated with the shift of the differential formula to the right is an increase in the disintegration index. This increase in the fragility of the platelets associated with a simultaneous increase in the larger types of thrombocytes would indicate that the larger forms disintegrate more readily than the smaller platelets. In a patient with bronchial asthma there was a slight decrease in the

disintegration index following the injection of adrenalin, associated, however, with an increase in the platelets of Groups I and III and a decrease in those of Group II. It would, therefore, seem that the sequence of disintegration of platelets is as represented in Fig. 1.

TABLE I

CHANGES IN THE TOTAL AND DIFFERENTIAL COUNTS RESULTING FROM THE DISINTEGRATION OF PLATELETS

PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
		GROUP I		GROUP II		GROUP III		GROUP IV			
0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
418,000	310,000	26.9	41.0	62.3	50.0	10.8	9.0	0.0	0.0	25.8	Normal
519,000	342,000	25.0	50.0	67.0	48.0	6.0	2.0	2.0	0.0	34.1	Normal
500,000	316,000	21.0	45.0	67.0	49.0	12.0	6.0	0.0	0.0	36.6	Normal
469,000	204,000	14.0	39.2	65.0	52.0	21.0	8.8	0.0	0.0	55.1	Normal
527,000	384,000	9.1	36.0	67.4	57.0	21.0	7.0	0.5	0.0	27.1	Normal
900,000	993,000	42.6	65.9	41.7	27.4	6.1	2.2	9.6	4.5	+10.3	Polycythemia vera (Case 9, Table IV)

TABLE II

CHANGES IN THE DISINTEGRATION INDEX, TOTAL AND DIFFERENTIAL PLATELET COUNTS FOLLOWING THE INJECTION OF ADRENALIN

	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
			GROUP I		GROUP II		GROUP III		GROUP IV			
	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
Before adrenalin injection	445,000	268,000	26.0	----	62.5	----	11.5	----	----	----	40.0	Normal
After adrenalin injection	604,000	106,000	21.9	----	70.1	----	8.0	----	----	----	82.4	
Before adrenalin injection	29,000	29,000	16.7	----	33.3	----	50.0	----	----	----	0.0	Chronic thrombocytopenic purpura
After adrenalin injection	50,000	31,000	0.0	----	60.0	----	40.0	----	----	----	38.0	
Before adrenalin injection	252,000	217,000	32.8	----	50.4	----	16.8	----	----	----	13.8	Aleucemic myelosis
After adrenalin injection	518,000	331,000	24.6	----	59.1	----	16.2	----	----	----	36.1	
Before adrenalin injection	252,000	152,000	25.0	----	55.0	----	20.0	----	----	----	39.6	Bronchial asthma (during seizure)
After adrenalin injection	407,000	253,000	27.8	----	46.7	----	25.5	----	----	----	37.8	
Before adrenalin injection	402,000	237,000	61.8	----	35.2	----	3.0	----	----	----	41.0	Catarrhal jaundice
After adrenalin injection	575,000	284,000	28.5	----	68.0	----	3.5	----	----	----	50.6	

Under abnormal conditions the number of small platelets at the end of the observation period may be very high. This may be due to a very rapid disintegration of the larger platelets or to a slow rate of disintegration of the smaller forms, or to a combination of both factors. Under these circumstances the disintegration index may be 0 or even greater, i.e., the final count may be equal to or greater than the initial count (a disintegration index greater than 0 is preceded by a + sign). In general, a disintegration index of less than 10 or greater

than 0 indicates abnormal stability of the platelets. However, a disintegration index of 0 based on numerical calculations implies that none of the platelets in the specimen of blood examined have disintegrated within the period of observation. This conception is incorrect unless the associated differential formula remains unchanged or nearly so, revealing none or only slight changes at the end of the observation period. This is illustrated by a patient with polycythemia vera (Table I) in whom the final count was greater than the initial count. Despite this increase in the total eight-hour count, the differential formula shows that 5.1 per cent of the platelets of Group IV, 3.9 per cent of Group III, and 14.3 per cent of Group II, or an absolute total of about 178,000 platelets per c.mm. have become disintegrated in the course of eight hours. A consideration of the changes in the differential platelet formula is, therefore, at times important in evaluating the results obtained following the disintegration of the thrombocytes.

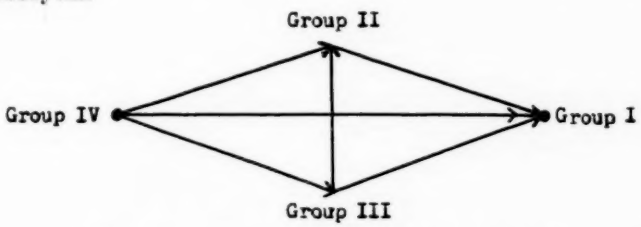

Fig. 1.—Sequence of disintegration of platelets.

THE RATE OF DISINTEGRATION OF PLATELETS IN DISEASE

Anemia.—In the chronic anemias, secondary and primary, and in the anemias due to acute blood loss, the disintegration index is normal (Table III); the platelets in these conditions disintegrate fairly rapidly. As the anemia improves with iron or liver therapy, the disintegration index tends to decrease. There is a simultaneous rise in the total platelet count to the normal level and a change in the differential formula characterized by a shift to the left due to an increase in the number of the smaller thrombocytes. In these conditions the long-standing thrombopenia, consisting of platelets of moderate fragility, is followed by a normal thrombocyte count consisting of platelets relatively more resistant to disintegration. The disintegration index eventually becomes normal, as illustrated by Case 5 who has been under liver treatment for a long time.

Polycythemia Vera.—Untreated cases of polycythemia vera usually yield normal disintegration indices (Cases 6 and 7, Table IV). Patients in Cases 8 and 9, treated with phenylhydrazine, yielded disintegration indices greater than 0, i. e., the final total platelet counts were greater than the initial counts. That some of the platelets have disintegrated is apparent from the fact that in Case 8 at least 7 per cent of the platelets of Group III and 9 per cent of Group I, or an absolute total of about 70,000 platelets per c.mm., have disappeared at the end of eight hours. Similarly in Case 9, 5.1 per cent of the platelets of Group IV, 3.9 per cent of the platelets of Group III and 14.3 per cent of Group II, or a total of about 178,000 platelets per c.mm. have disintegrated. However, even when considered from this aspect, the rate of disintegration of the platelets in these polycythemics treated with phenylhydrazine is rather slow.

This is in agreement with the observations of Morawitz and Pratt[34] and Itami and Pratt[35] who noted an increased resistance of the red cells in experimental phenylhydrazine anemias.

TABLE III

DISINTEGRATION INDEX IN ANEMIAS

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
1	M	33	250,000	136,000	29.2	48.0	39.8	48.0	27.7	4.0	3.3	0	45.6	Chronic bleeding peptic ulcer; R.B.C. 2.29 mil.
			488,000	440,000	36.4	32.0	50.0	56.0	12.5	12.0	1.1	0	9.8	One month later; R.B.C. 5.42 mil.
2	M	55	674,000	490,000	60.0	42.0	35.0	58.0	5.0	0.0	0.0	0	27.3	Acute bleeding peptic ulcer.
3	M	56	293,000	193,000	14.7	62.0	55.4	34.0	27.4	4.0	2.5	0	34.1	Primary hypochromic anemia.
			451,000	332,000	22.7	34.0	56.4	60.0	20.0	6.0	0.9	0	26.3	After a month's treatment with iron.
4	M	68	398,000	213,000	33.3	32.0	42.0	60.0	24.7	8.0	0.0	0	46.5	Pernicious anemia.
			508,000	364,000	32.2	55.0	47.1	38.0	20.7	7.0	0.0	0	28.3	After 6 weeks of liver treatment.
5	M	37	421,000	226,000	19.4	41.0	60.5	46.0	20.1	13.0	0.0	0	46.3	Pernicious anemia under liver treatment for a long time.

TABLE IV

DISINTEGRATION INDEX IN POLYCYTHEMIA VERA

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	REMARKS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
6	F	50	581,000	377,000	25.8	34.0	41.0	50.0	29.0	16.0	4.2	0	35.1	Untreated
7	M	46	714,000	492,000	58.7	63.0	37.3	36.0	1.6	1.0	2.3	0	31.1	Untreated
8	M	60	462,000	470,000	25.0	16.0	40.0	56.0	35.0	28.0	0.0	0	+ 1.7	Under phenylhydrazine treatment.
9	F	50	900,000	993,000	42.6	65.9	41.7	27.4	6.1	2.2	9.6	4.5	+10.3	Under phenylhydrazine treatment.

Phenylhydrazine, when administered to patients with polycythemia vera, is well known to cause at times enormous increases in the number of circulating platelets. According to Jürgens and Bach[36] this phenylhydrazine thrombo-

cytosis constitutes an important predisposing factor in the causation of spontaneous thrombosis and embolism in these patients. However, the rather slow rate of disintegration of platelets during this form of therapy tends to counteract the thrombophilia. It is perhaps for this reason that spontaneous thrombosis in polycythemics treated with phenylhydrazine is not seen more frequently.

Essential Thrombocytopenic Purpura.—The disintegration index was 0 in two patients presenting marked thrombopenia (Cases 11 and 12, Table V). Moderate thrombopenia, however, may show a normal rate of disintegration. This is shown by Case 10, Table V; the patient had an extremely abnormal

TABLE V

DISINTEGRATION INDEX IN CHRONIC THROMBOCYTOPENIC PURPURA

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	REMARKS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
*10	M	25	127,000	88,000	1.0	2.0	15.0	6.0	84.0	92.0	0	0	30.7	
			406,000	322,000	5.5	2.0	22.6	18.0	71.9	80.0	0	0	20.7	After three blood transfusions.
11	F	27	55,000	55,000	50.0	--	40.0	---	10.0	---	--	--	0	
12	F	31	29,000	29,000	16.7	--	33.3	---	50.0	---	--	--	0	

*The increase in the number of platelets in Group III at the end of eight hours is due to the fact that many of the platelets were the size of a red cell or even larger. The disintegration products of these macrothrombocytes at the end of the observation period were sufficiently large to be placed in this group.

differential formula with numerous large and giant platelets, many of them the size of a red cell. Following three blood transfusions the disintegration index dropped from 30.7 to 20.7, the total count in the meantime having risen from 127,000 to 406,000 platelets per c.mm. with the differential formula exhibiting a shift to the left.

Hemophilia.—The rate of disintegration of the platelets was fairly rapid in a patient with hemophilia (Table VI) who exhibited normal total and differ-

TABLE VI

DISINTEGRATION INDEX IN HEMOPHILIA

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.
					GROUP I		GROUP II		GROUP III		GROUP IV		
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	
13	M	13	541,000	284,000	18.8	16.8	60.0	61.9	19.4	20.7	1.9	0.6	47.5

ential platelet counts. The eight-hour differential formula, however, revealed relatively slight changes. In other words, the normally more resistant platelets of Group I and the less resistant platelets of the other groups all disintegrated at approximately the same rate.

It has been frequently stated that in hemophilia the platelets are resistant to disintegration (Stübel,[3] Fonio,[37] Minot and Lee,[38] Howell and Cekada[39]); the thrombocytes, being usually stable, fail to yield readily the platelet factor essential for blood coagulation. Birch[40] observed that by mechanically traumatizing the platelets hemophilic blood could be made to clot normally. Howell,[41] Minot, Denny and Davis[42] and Hurwitz and Lucas[43] noted a reduction in the

amount of prothrombin in hemophilia. This would also seem to depend on the slow disintegration of the platelets since, according to Bayne-Jones[44] and Tait and Green,[45] prothrombin and unruptured thrombocytes constitute the same substances. On the other hand, Feissly and Fried,[46] Fieschi[47] and Eagle[48] maintain that the hemophilic platelets are fundamentally normal. Moreover, from my own observations and from those of Baar and Székely,[17] it would appear that hemophilic platelets possess normal fragility. As a matter of fact there is considerable evidence indicating that a number of other factors are involved in the pathogenesis of the hemorrhagic tendencies in hemophilia. Birch,[13, 49] for example, found entire lack of female sex hormone in the urine of these patients. Feissly and Fried[46] and Fieschi[47] observed that in hemophilia the plasma is abnormal, since they failed to observe any difference in the coagulation time of normal plasma upon adding platelets from normal and from hemophilic subjects. Bernuth[50] noted that, in some cases of hemophilia, the cut capillaries failed to disappear by contraction in a normal manner, remaining visible for a long time. Stuber and Lang[51] found that in this disease there is an excess of fluoride in the blood which brings about a prolongation of coagulation by retarding glycolysis of the blood cells. These same investigators[52] also observed that in hemophilia the negative electrical charge of the platelets is increased; this causes an increase in their reciprocal electrostatic repulsion and a decrease in their rate of agglutination.

Leucemia.—Normal disintegration indices were observed in a patient with chronic myelogenous leucemia (Case 14, Table VII) and in one with chronic

TABLE VII

DISINTEGRATION INDEX IN LEUCEMIAS

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR		
14	M	46	736,000	414,000	37.8	54.2	45.4	43.4	5.9	2.4	10.9	0	43.7	Chronic myelogenous leucemia
			548,000	298,000	45.8	49.0	46.4	49.0	7.8	2.0	0.0	0	45.6	After x-ray treatment
15	F	42	252,000	217,000	32.8	40.0	50.4	54.0	16.8	6.0	0.0	0	13.8	Aleucemic myelosis
16	M	38	465,000	321,000	9.0	36.0	64.5	56.0	24.0	8.0	2.5	0	30.9	Chronic lymphatic leucemia

lymphatic leucemia (Case 16). In a patient with aleucemic myelosis (Case 15) the thrombopenia noted was associated with a rather low disintegration index.

Malignancy.—The disintegration index is essentially normal in malignancy (Table VIII) and does not appear to be influenced by the total or the differential platelet counts. In Cases 22 and 23, following therapeutic roentgen ray irradiation, there was a drop in the disintegration index associated with a simultaneous decrease in the total count and also a change in the differential formula characterized by a shift to the left. In a patient with chronic myelogenous

TABLE VIII

DISINTEGRATION INDEX IN MALIGNANCY

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
17	M	55	638,000	459,000	47.0	64.0	34.0	33.0	7.0	3.0	12.0	0	28.0	Cancer of colon
18	M	57	721,000	493,000	16.0	47.0	49.0	47.0	35.0	6.0	0.0	0	31.6	Cancer of stomach
19	F	52	962,000	631,000	65.7	68.7	29.1	30.5	3.1	0.8	2.1	0	34.4	Cancer of stomach
20	F	50	385,000	291,000	12.0	30.0	40.0	59.0	48.0	11.0	0.0	0	24.4	Cancer of neck
21	F	59	601,000	491,000	56.0	60.0	36.8	40.0	5.6	0.0	1.6	0	18.3	Cancer of breast
22	M	60	607,000	347,000	12.4	26.0	61.4	60.0	24.8	14.0	1.4	0	42.8	Cancer of lung
			469,000	434,000	45.1	65.0	45.9	30.0	9.0	5.0	0.0	0	7.4	After x-ray treatment
23	M	38	677,000	410,000	23.6	50.0	54.8	42.0	21.6	8.0	0.0	0	39.4	Hodgkin's disease
			627,000	414,000	35.5	58.0	47.8	30.0	16.7	12.0	0.0	0	34.0	After x-ray treatment
			702,000	574,000	62.1	60.0	29.3	38.0	2.3	2.0	6.3	0	18.2	Two months later

leucemia (Case 14, Table VII) there was only a slight change in the disintegration index following roentgen ray irradiation. Here, however, the decrease in the total count was associated with only slight changes in the differential platelet formula.

Infection.—In chronic infections the disintegration index is normal when the associated platelet count is normal or elevated (Cases 25, 26, 27, Table IX). The disintegration index may be low in the presence of thrombopenia (Case

TABLE IX

DISINTEGRATION INDEX IN INFECTION

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
24	M	46	320,000	290,000	10.0	---	60.7	---	29.3	--	0.0	--	9.4	Acute stage of lobar pneumonia
			1,344,000	866,000	52.6	76.6	37.9	23.4	5.1	0.0	4.5	0.0	35.5	During convalescence
			427,000	439,000	63.5	64.0	32.7	35.0	3.8	1.0	0.0	0.0	+2.8	Patient well
25	M	55	611,000	239,000	62.5	80.0	31.0	20.0	1.9	0.0	4.6	0.0	60.0	Bronchiectasis
26	F	40	658,000	472,000	57.8	80.0	37.1	20.0	3.1	0.0	3.1	0.0	28.2	Active pulmonary tuberculosis
27	M	31	478,000	366,000	26.3	30.0	50.0	62.0	23.7	8.0	0.0	0.0	23.4	Subacute bacterial endocarditis
28	M	21	204,000	199,000	50.0	56.0	40.0	38.0	6.0	0.0	6.0	2.4	2.4	Subacute bacterial endocarditis

28). In acute infections (Case 24, Table IX) the disintegration index is low when thrombopenia exists, rises during convalescence when thrombocytosis appears, and then decreases as the normal level is reached. During the latter stage the slow disintegration of the platelets is apparently due to the presence of relatively numerous young, resistant thrombocytes as manifested by the differential formula.

Postoperative Behavior of the Disintegration Index.—Following surgical operations there is usually a decrease in the total platelet count during the first twenty-four hours. This is followed by a gradual increase in the number of platelets reaching a maximum level toward the end of the second week, and then gradually returning to normal. The entire cycle occupies about three weeks. The disintegration index exhibits a corresponding swing (Table X,

TABLE X

POSTOPERATIVE BEHAVIOR OF DISINTEGRATION INDEX

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
29	F	41	342,000	305,000	43.0	65.0	51.0	33.0	6.0	2.0	0.0	0.0	10.8	Cholecystectomy 24 hours postoperatively
			879,000	600,000	59.2	71.8	30.2	21.2	7.0	4.0	3.3	3.3	31.7	Sixteenth postoperative day
30	F	45	536,000	486,000	78.0	80.0	20.9	19.5	1.1	0.5	0.0	0.0	9.5	Amputation of foot 12 hours postoperatively
			901,000	513,000	69.2	80.0	22.8	16.0	4.0	2.0	2.0	1.0	43.1	Thirteenth postoperative day

Chart 3). It is low during the early postoperative period, rises as the total platelet count increases, then decreases as the normal platelet level is reached. During the stage of thrombocytosis more than half of all the platelets belong to Group I. However, despite the presence of many young, relatively resistant platelets, the disintegration index is fairly high. This is probably due to the fact that the remaining platelets are unusually fragile. These observations are in accord with those of König[23] who noted that postoperatively the platelets, owing to the damaging effect of the spleen, disintegrate more readily than normally.

The rate of disintegration of the platelets is probably a factor of importance in the causation of spontaneous venous thrombosis as observed postoperatively and also during the course of other diseases. Spontaneous venous thrombosis is nearly always associated with an elevated platelet count in conditions such as postoperative states (Hueck[53-55]), fractures (Galloway[56]), parturition (Dawbarn, Earlam and Evans[57]), severe acute hemorrhage (Jagic and Klima[58]), infection (Brock and Rake[59]), certain splenic anemias (Rosenthal[60]), malignancy (Naegeli[61]), and polycythemia vera (Jürgens and Bach[36]). Essential thrombo-

philia described by Nygaard and Brown[62] constitutes an exception, for in this disease the platelet count is normal. The characteristic features of this thrombocytosis are moderate fragility and increased agglutinability of the platelets. It is, therefore, probable that the rapid disintegration of the thrombocytes plays an important rôle in the pathogenesis of spontaneous intravascular clotting. In fact, König[23] maintains that the decreased stability of the platelets is a very significant predisposing factor favoring the development of spontaneous venous thrombosis and embolism during postoperative states.

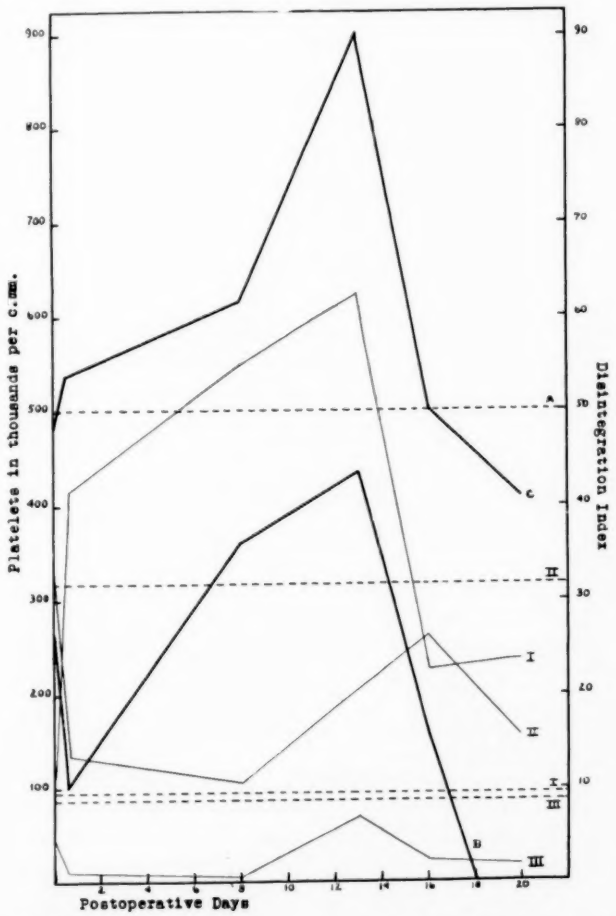

Chart 3.—Behavior of the disintegration index and the differential platelet formula postoperatively (following amputation of foot, Case 30, Table X). Dotted line *A* represents the normal platelet level, dotted lines *I*, *II*, and *III* the normal absolute levels of the corresponding groups. Continuous line *C* represents the behavior of the total platelet count, continuous lines *I*, *II*, and *III* the absolute counts of the corresponding groups. Continuous line *B* represents the behavior of the disintegration index.

Miscellaneous Diseases.—The fragility of the platelets was normal in a patient with purpura rheumatica (Case 31, Table XI), and in one with hemochromatosis (Case 40). The latter patient presented an extremely abnormal differential formula due to the presence of numerous large and giant platelets. The disintegration index was normal in patients with jaundice (Cases 35, 36, 37, 38, 39, Table XI) despite the frequently encountered thrombopenia asso-

ciated with an abnormal differential formula revealing a shift to the left. The platelets appeared to be abnormally resistant to disintegration in a patient with senile purpura (Case 32), in one with scurvy (Case 33), and in one with malignant nephrosclerosis (Case 34). The last patient presented numerous spontaneous hemorrhages from the mucous membranes of the mouth and skin.

TABLE XI

DISINTEGRATION INDEX IN MISCELLANEOUS DISEASES

CASE	SEX	AGE	PLATELETS PER C.MM.		DIFFERENTIAL PLATELET FORMULA (PER CENT)								D. I.	DIAGNOSIS
					GROUP I		GROUP II		GROUP III		GROUP IV			
			0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.	0 HR.	8 HR.		
31	F	32	471,000	285,000	15.3	29.0	70.3	61.0	13.7	10.0	0.7	0.0	39.5	Purpura rheumatica
32	M	70	355,000	415,000	9.3	37.0	42.6	53.0	47.4	10.0	0.7	0.0	+17.0	Senile purpura
33	F	50	423,000	410,000	70.9	70.0	25.2	28.0	3.9	2.0	0.0	0.0	3.0	Scurvy (partly treated)
34	F	30	300,000	287,000	24.0	38.0	47.0	50.0	27.0	8.0	2.0	4.0	4.3	Malignant nephrosclerosis
35	F	35	255,000	157,000	36.6	43.0	35.8	50.0	24.4	7.0	3.2	0.0	38.4	Catarrhal jaundice
			437,000	371,000	12.7	14.0	68.6	70.0	18.7	16.0	0.0	0.0	15.1	Patient well
36	M	31	402,000	237,000	61.8	56.0	35.2	40.0	3.0	4.0	0.0	0.0	41.0	Catarrhal jaundice
37	M	30	345,000	268,000	25.0	39.0	56.0	49.0	19.0	12.0	0.0	0.0	22.3	Catarrhal jaundice
38	M	23	406,000	293,000	31.0	28.0	41.0	54.0	28.0	8.0	0.0	0.0	27.8	Chronic hemolytic jaundice
39	F	28	367,000	279,000	33.0	50.0	53.0	45.0	14.0	5.0	0.0	0.0	31.5	Jaundice due to arsphenamine hepatitis
40	M	59	257,000	174,000	4.5	18.0	39.1	48.0	54.3	34.0	2.1	0.0	32.3	Hemochromatosis

EFFECT OF ROENTGEN RAY IRRADIATION ON THE BLOOD PLATELETS

Observations on the effect of roentgen ray irradiation on the circulating blood platelets have yielded rather confusing results. Helber and Linser[63] and Haenisch and Holthusen[64] maintain that roentgen rays have a slight effect on the platelets. Gaviati,[65] on the other hand, is of the opinion that the platelets are very sensitive to x-ray irradiation. Falconer, Morris, and Ruggles[66] noted that direct x-ray irradiation of the long bones, in both light and heavy dosage, did not appreciably stimulate or decrease the contained marrow cells. According to Wittkower[67] there may be an increase or decrease in the number of circulating platelets after x-ray irradiation. Henkel and Gueffroy[68] observed no change in the number of platelets after roentgen ray exposure. According to Minot and Spurling[69] in malignancy medium x-ray doses cause a slight increase in the number of circulating platelets, larger doses a great increase ever up to 100 per cent, larger doses still after an initial increase produce a decrease, yet the count remains above normal. Hittmair, Luze and Hönlinger[70] noted an increase in the platelet count to over a million per cubic millimeter following

irradiation for lymphogranulomatosis. Many investigators (Falconer, Morris and Ruggles,[66] Schintz,[71] Hittmair[72] among others) have observed an increase in the number of platelets following irradiation of the spleen. I have failed to observe this reaction in two patients with purpura hemorrhagica. On the other hand, severe thrombopenias may be produced in animals by exposing them to roentgen rays (Helber and Linser,[63] Duke,[73] Fabricius-Möller[74]). In human subjects decreases in the platelet count after x-ray irradiation have been observed by Schintz,[71] Frei and Alder,[75] and Hirschfeld and Hittmair.[76] Apparently the biologic reactions following exposure to radium are essentially the same (Cramer, Drew and Mottram[77]).

There is no doubt that the reaction on the part of the platelets depends on the condition of the patient, the type of apparatus employed and the amount of irradiation. One very significant factor is the absolute platelet level. There is nearly always a reduction in the number of circulating platelets following therapeutic doses of roentgen ray irradiation in patients presenting moderate or marked thrombocytosis. I have observed this reaction in malignancy, Hodgkin's disease, chronic myelogenous leucemia, and polycythemia vera.

No observations are recorded in the literature concerning the effect of x-ray irradiation or radium on the fragility of platelets. From my own observations, it would appear that the rate of disintegration of the platelets is decreased following therapeutic roentgen ray irradiation whenever there is a shift of the differential formula to the left due to an increase in the number of small platelets. These observations also suggest that the larger, more mature platelets are more sensitive to roentgen rays than the smaller thrombocytes.

SUMMARY AND CONCLUSIONS

1. A method is described for the simultaneous determination of the rate of disintegration, total and differential counts of the platelets.

2. The large, more mature platelets are less stable and disintegrate more readily than the small, juvenile thrombocytes.

3. The rate of disintegration of the platelets varies considerably in normal persons. In a group of 26 normal individuals the following results were obtained: in 50 per cent of the subjects, 21 to 40 per cent of all the platelets disintegrated in the course of eight hours, and in 85 per cent, 11 to 60 per cent of the platelets became completely disintegrated during the observation period. Abnormal stability of the platelets is indicated by a slow disintegration rate when 10 per cent or less of all the thrombocytes become disintegrated during the observation period. Abnormally rapid disintegration rates, when 60 per cent or more of the platelets disintegrate in the course of eight hours, are very infrequent in normal or abnormal states.

4. The rate of disintegration of the platelets was studied in a variety of diseases. In chronic conditions such as anemia, at times essential purpura, purpura rheumatica, hemophilia, polycythemia vera, leucemia, malignancy, jaundice, hemochromatosis, infection and postoperative states the fragility of the platelets was normal and did not appear to be influenced by the total thrombocyte level or the differential platelet formula. As the total platelet count changed there was a simultaneous change in the stability of the platelets usu-

ally associated with a shift of the differential formula to the left. As a rule the fluctuations remained within normal limits. However, it was the relative variations in the rate of disintegration of the platelets that appeared to be of significance. A change in the rate of the disintegration of the thrombocytes from 9.5 per cent to 43.1 per cent (Case 30, Table X), for example, indicates a change in the resistance of the platelets from a state of moderate stability to one of moderate fragility despite the fact that these values fall within the normal range. In thrombopenic states the rate of disintegration of the platelets decreased as the total platelet count rose to the normal level. In conditions associated with thrombocytosis (infective and postoperative states) the fragility of the platelets increased as the total platelet count rose and then decreased as the normal platelet level was reached. The increase in the fragility of the platelets during infective and postoperative states, though relative, is probably of considerable significance in the pathogenesis of spontaneous venous thrombosis at times observed in these conditions.

5. Abnormal stability of the platelets was noted in marked and occasionally in moderate thrombopenias, in scurvy, senile purpura, malignant nephrosclerosis, polycythemia vera treated with phenylhydrazine, and at times in conditions with normal platelet counts following a recent preexisting thrombocytosis (infective and postoperative states). This abnormally high resistance of the platelets was frequently associated with spontaneous hemorrhages.

6. Following therapeutic roentgen ray irradiation there was an increase in the resistance of the platelets to disintegration associated with a simultaneous decrease in their number and a change in the differential formula characterized by a shift to the left due to an absolute increase in the number of the smaller thrombocytes.

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THE VALUE OF AIR-CONDITIONED ROOMS IN THE TREATMENT OF SEASONAL AND PERENNIAL ASTHMA*

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AIR conditioning has decided therapeutic values. This has been proved by many investigators in the field of Sanitary Engineering, and accepted by medical authorities. Its application is not only limited to the sickroom or the hospital, but it offers comfort for the healthy one.

The purpose of this presentation is to record our observations of the effects in the treatment of seasonal and nonseasonal asthma.

The Mount Sinai Hospital has two rooms equipped with built-in room type units. Each room contains four beds about 3,200 cu. ft. of air space.

The units which are classed as HC600 units were built and put in operation April, 1934. Each unit is capable of delivering 400 cubic feet of air per minute at a dry bulb temperature of 60°, a dew point of 55° and of maintaining a room temperature at 80° dry bulb and 67° wet bulb with outside conditions 95° dry bulb and 77° wet bulb. We maintain a room temperature of from 73 to 74° dry bulb and from 64 to 66° wet bulb. This gives a relative humidity of 63 per cent with eight complete air changes per minute. The operation of the unit is as follows: There is 20 per cent air drawn in from the outside which passes to the bottom where it mixes with the air being drawn in from the room. This mixed air passes up through refrigeration coils where it is cooled and dehumidified and then through a filter which removes pollen and dust, and then through a fan which circulates the air over steam coils. Then the air is blown into the room. The refrigeration and steam are automatically controlled by solenoid valves, thermostat and humidstat, and the air is humidified in winter by the air passing through a water spray. The compressors which are installed in the basement have a capacity of one and one-half tons of refrigeration and are charged with a compound known as F12 or Feon which is dichlorodifluoromethane and is nonpoisonous, colorless, odorless, and noncombustible except under extreme temperature. The compressor is driven by a two-horse power motor.

All of our asthmatics are usually admitted to these wards, where they are carefully observed and studied. The number of admissions during this period totals 40.

For practical purposes our cases can be divided into three groups: (1) Pollen asthma; (2) perennial asthma; (3) mixed group, i.e., perennial asthmatics who have definite seasonal aggravation usually due to pollen.

Our pollen asthma cases totaled 10, 7 ragweed and 3 timothy. One case, while seasonal in incidence, had no definite etiology.

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During the summer of 1934, we had 3 cases, and in 1935, 8 cases. All were asthmatics. Two also had nasal symptoms. Eight did not receive any preseasonal treatment. Two began coseasonal treatment and failed to get any relief within one week. They were admitted in the height of the season, and all of them were in a very severe form of "status asthmaticus." All of them, except one, showed evidence of improvement within two to three hours after admission, and marked improvement within twenty-four hours. One of the cases was practically moribund. She was admitted late on the night of September 4. During ward rounds about 10 A.M. the following morning, she was very comfortable, although her chest was still barrel-shaped and filled with various musical râles.

The one exceptional case that failed to respond in 1934 and 1935 is quite a puzzle to us. He is an Armenian of about fifty-two years, who is engaged in the industry of cleaning and repairing of rugs. His duties, however, are limited to collecting and delivering only. His symptoms are of twenty years' duration, and are always limited to July and the early part of August. Skin tests and conjunctival tests were negative to all of the known midsummer pollens. The only moderate reaction obtained was to house dust. We were unsuccessful in bringing on an attack of asthma by introducing powdered pollen into the nasal chamber and conjunctival sac. The clinical course is usually associated with fever, ranging from 100 to 101° F., and the physical signs, in addition to the typical picture of asthma, areas of bronchopneumonia; x-ray of the chest on three different occasions failed to reveal any consolidation. It was not associated with hemoptysis. Sputum examination revealed a number of eosinophiles. The blood examination revealed a leucocytosis of about 15,000 with a normal differential containing 3 or 4 per cent eosinophiles.

What the cause of this seasonal asthma is remains undecided.

Kahn and Grothaus[1] feel that air-conditioned rooms have not only a therapeutic but also a diagnostic value. They feel that when relief in air-conditioned rooms is obtained in patients who give negative skin reactions to pollen, they call it "cutaneous anergy," it is proof that pollen was the cause of the asthma. If the reverse is true, then our exceptional case is seemingly not a pollen asthmatic.

In order to demonstrate that the beneficial results in the above cases were due to the air-conditioning, may we cite the cases of two patients admitted to the private service of A. T. in the summer of 1933.

These two patients were victims of timothy allergy. Both were untreated and in "status asthmaticus." They were admitted about the middle of July. It took more than twenty-four hours before their attacks could be ameliorated, and then they never remained symptom-free, requiring epinephrin and other similar medications to control frequent attacks. While we made no pollen counts, we recollect definitely that during a rainy day they would invariably feel better, and attacks would recur whenever wind and sunshine prevailed.

The "mixed group" consists of seven patients. It may be of interest to note that two began with seasonal asthma, and one with seasonal hay fever before they developed perennial symptoms. All of them had preseasonal pollen treatment as well as treatment for the perennial factors without any relief. They were admitted during the latter part of their respective pollination season, and

all of them were in "status asthmaticus." No relief was noted within twenty-four hours, and in a few, in forty-eight hours. Only one patient, who had a rather mild form of perennial asthma, developed very severe symptoms during the timothy season. He was relieved within a few hours after admission, so that all medications were discontinued after the first day of hospitalization. He was discharged at the end of July, and enjoyed good health until the middle of September, when he contracted an acute upper respiratory infection, resulting in a severe form of asthma. Readmission this time did not yield any dramatic results. Improvement of his asthma followed the improvement of his upper respiratory infection, which took about two or three days.

The perennial asthma group consists of 22 cases. They reacted to various inhalants and ingestants. A few had chronic pathology in the paranasal sinuses. Five of this group were admitted in "status asthmaticus." The remainder were admitted either for additional studies, nasal surgery, or to observe the effects of environmental changes.

The immediate effects were not dramatic. It took twenty-four and forty-eight hours before any relief was definitely obtained. Those in "status asthmaticus" had to have epinephrine every three hours, and other emergency measures in order to be free from attacks. The others had to have nightly administration of epinephrine. All of them, however, experienced some relief within a few days.

Four patients who were admitted to the general wards experienced just as much relief within two days. Two patients, one a woman of sixty years, and the other twenty-eight, were worse in the air-conditioned room and begged to be removed to the general ward.

This again is in conformity with the experience of Gay,[2] Nelson, Rappoport and Walker.[3]

COMMENT

Our results confirm the observations of Gay who was able to obtain favorable results in his asthmatic patients within one hour after their admission to the air-conditioned room, and that of Nelson, Rappoport and Walker, who report 50 per cent improvement in their asthmatic patients within three hours or less. While the bulk of their patients consisted of hay fever cases whose symptoms were referable to the upper respiratory tract, we feel that the asthmatic patients surely present a much more urgent problem in treatment; hence, the need of air conditioning is greater.

It is also evident from our results that only certain extrinsic forms of asthma are likely to benefit from this method of treatment. One should not expect to obtain relief in such an atmosphere when sensitivity exists to the animal danders, which are contained in that room. This is well exemplified in the case of Vaughan and Cooley[4] whose patient was sensitive to feathers, and failed to improve in the air-conditioned room which contained feathers. Intrinsic factors are not controlled nor removed. As in the case of Gay, patients suffering from bacterial allergy cannot expect any benefit in such rooms. In fact, they are frequently made very uncomfortable. This explains the failure of treatment in the perennial as well as mixed asthmatic patients in air-conditioned atmospheres.

Where environmental factors exist, one does not need air-conditioned rooms. Such patients will get just as much relief in the general wards. It is our experience, and we are sure, that of every allergist, that we obtain marked relief in such patients upon their admission to the hospital, and that they stay well while in the hospital, and develop seizures upon returning to their old environment.

We are inclined to agree with Kahn and Grothaus that the air-conditioned room has some diagnostic value. Failure to obtain immediate relief in such an atmosphere would justify the exclusion of pollen as a factor.

SUMMARY

1. Air-conditioned atmosphere has a definite value in the treatment of seasonal asthma, due to pollen.

2. It is useless in other forms of asthma, in fact, it may aggravate cases of bacterial allergy.

3. The improvement obtained in the nonseasonal asthmatic is usually due to the removal of some environmental factors to which the patient is sensitive. Such results can easily be obtained in the general wards which are not air-conditioned.

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PRIMARY CARCINOMA OF THE THYMUS GLAND*

Case Report

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PRIMARY carcinoma of the thymus gland is a relatively rare tumor. Crosby,[1] in an exhaustive review of the literature on thymogenic tumors, was able to collect reports on 44 cases of thymic carcinoma out of a total of 166 cases of primary thymic tumors. We have been able to find reports of 9 other cases since Crosby made his summary. Symmers[2] reported 2 cases. Leyton, Turnbull, and Bratton[8] reported 2 cases. McDonald,[3] Demel,[4] Touroff,[5] and Collins,[6] each reported 1 case. These, together with our own case, bring the total number up to 53.

Symmers[2] subdivides thymic tumors into the following groups, in order of their frequency: (1) Lymphosarcoma, (2) perithelioma, (3) Hodgkin's disease, (4) epithelioma, and (5) spindle-cell sarcoma. He reported a total of 25 primary thymic tumors, of which 2 were epitheliomas.

Ewing[7] gives the following classification: (1) Lymphosarcoma or thymoma, consisting of a diffuse growth of round, polyhedral, and giant cells; (2) carcinoma; and (3) spindle-cell sarcoma or myxosarcoma.

The occurrence of epithelial tumors of the thymus gland can be explained on an embryologic basis. The gland has its origin from the entodermal pouches that arise from the third and fourth branchial clefts. The reticulum cells and Hassal's corpuscles are of epithelial origin. The epithelioma may arise either from Hassal's corpuscles or from the reticulum cells.

The clinical picture of all thymic tumors is the same. There is nothing to distinguish a carcinoma from a lymphosarcoma clinically, except that the latter sometimes responds to radiotherapy.

The subjective symptoms are those of pressure in the upper portion of the mediastinum. Cough, hoarseness, dyspnea, cyanosis, and edema of the neck and face are the chief symptoms. Exophthalmos is frequently encountered. The veins of the neck become distended. There may be some difficulty in swallowing.

Physical examination may reveal retrosternal dullness. A pericardial effusion is sometimes encountered. X-ray examination shows a tumor which is located in the upper portion of the anterior mediastinum.

The symptoms usually do not appear until quite late. In most of the cases that have been reported, the patient did not seek any medical advice until a few weeks or at the most a few months before death occurred. In our case the

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patient consulted a physician about two months before he died, and he stated that he had been perfectly well until about four weeks before that time.

A peculiar feature of some thymic tumors, particularly of the lymphosarcomas, is the similarity of the symptoms to those of myasthenia gravis. Several cases of this type have been reported. Leyton, Turnbull, and Bratton[8] reported 2 cases with pluriglandular disturbances; both of these cases were carcinomas.

The treatment of thymic tumors is unsatisfactory. Some of the lymphosarcomas and some of the cases of Hodgkin's disease respond, at least temporarily, to x-ray treatment. Carcinoma of the thymus gland is not radiosensitive.

Fig. 1.—Roentgenogram of chest.

REPORT OF CASE

F. M., white, male, aged forty-nine years. Admitted to the Windber Hospital on Oct. 30, 1934. Chief complaints were dyspnea and swelling of face and neck. He stated that he had been perfectly well until Oct. 1, 1934. At that time he noticed a moderate degree of dyspnea. About one week later he noticed some puffiness of his neck. The dyspnea became worse and he came to the hospital on October 30.

Physical Examination.—Patient was well nourished. There was a moderate degree of swelling of the face and neck. The lips were somewhat cyanotic. There was moderate exophthalmos of both eyes. The pupils were equal and reacted to light and accommodation. The external jugular veins were prominent on both sides. The respiratory excursion was diminished bilaterally. There was dullness to the right of the sternum. The heart sounds were distant.

X-ray Examination.—X-ray of the chest showed a large shadow in the upper portion of the mediastinum.

A diagnosis of mediastinal tumor was made, and the patient was given a course of deep x-ray treatments. However, he did not seem to respond to the treatment. His condition gradually became worse. He developed marked hoarseness. The edema of the face and neck became worse. Cyanosis became marked. He also developed marked edema of the scrotum and lower extremities. Death occurred on Dec. 26, 1934.

Autopsy.—The body was that of a well-nourished middle-aged male. The face, neck, scrotum, and lower extremities were edematous. Both eyes showed a moderate degree of

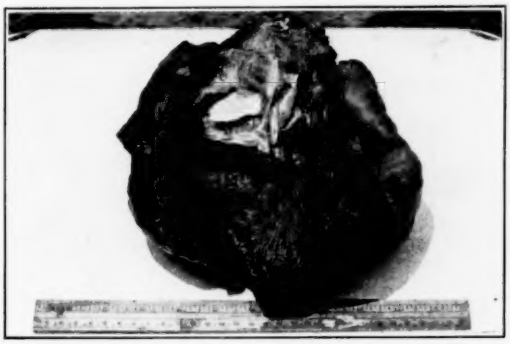

Fig. 2.—Gross appearance of tumor showing relationship to heart and lungs.

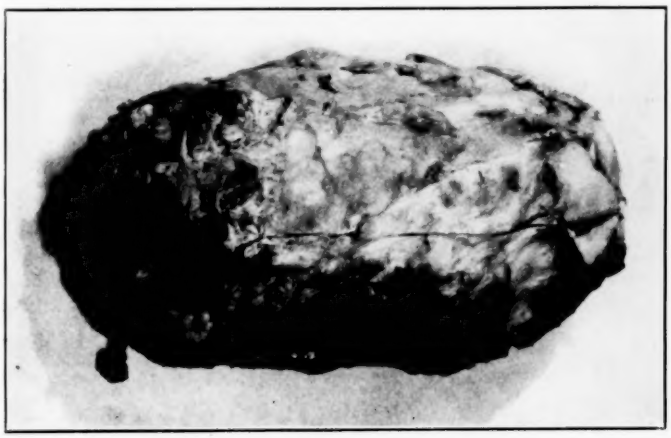

Fig. 3.—Gross appearance of tumor.

exophthalmos. The superficial veins of the neck were prominent. Upon opening the thoracic cavity, a large tumor was found in the upper portion of the anterior mediastinum. It occupied the position which is normally assigned to the thymus gland, and its contour was that of a large thymus gland, consisting of two lobes, the lower ends of which were separated by a notch. The tumor extended from the manubrium sterni to a point between the fourth and fifth ribs. It was extremely hard and was adherent to the surrounding tissues.

The pericardium contained about 125 c.c. of a straw-colored fluid. There were adhesions of the pleura at both apices. Both lungs showed a marked degree of pneumokoniosis. The azygos vein was markedly dilated as were also the pulmonary veins and pulmonary artery. There was a large thrombus in the right auricle. The peribronchial lymph nodes were en-

larged and hardened. Abdominal examination was essentially negative. There was no free fluid in the peritoneal cavity. There was no evidence of metastasis of the tumor to any of the abdominal organs.

Description of the Tumor.—

Gross Description: The tumor was somewhat grayish in color and was extremely hard. It weighed 525 gm. It measured 15½ cm. in length; its width varied from 4 cm. to 8 cm. It seemed to consist of two lobes, having the general contour of a thymus gland, and the lower end presented a notch. The cut surface had a white glistening appearance.

Microscopic Description: Histologic examination of the tumor showed a dense fibrous stroma in which were embedded large numbers of epithelial cells and a small number of lymphoblasts. The epithelial cells occurred singly and in small nests. In some areas there was a tendency to alveolar arrangement of the cells. Most of the epithelial cells were cuboidal or cylindrical. The nuclei were large and vesicular and showed many mitotic figures. The cytoplasm was pale.

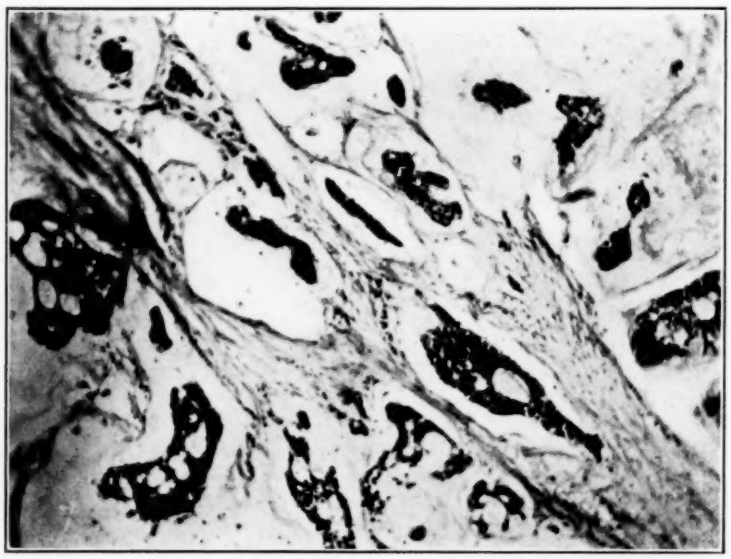

Fig. 4.—Microscopic appearance of tumor showing tendency to take on alveolar arrangement.

COMMENT

This tumor presents the outstanding characteristics which are used as criteria for the diagnosis of primary carcinoma of the thymus gland. These are as follows:

1. *Location:* That normally occupied by the thymus gland.

2. *Shape:* The contour of the thymus gland is simulated and there is a notch at the lower end.

3. *Structure:* The histologic structure is that of an epitheliomatous growth in a dense fibrous stroma. Lymphoid tissue is also present.

4. There is no evidence of any primary tumor elsewhere in the body which may have metastasized to the thymus.

Most thymic tumors have the above characteristics, with the exception that the histologic structure varies with the origin of the tumor.

SUMMARY

1. A case of primary carcinoma of the thymus gland is presented.

2. Dyspnea, cyanosis, swelling of the neck and face, and exophthalmos were the outstanding symptoms.

3. The first symptoms were noted less than three months before the patient died.

4. The tumor was not radiosensitive.

5. A typical carcinomatous structure was found upon histologic examination.

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FEVER ACCOMPANYING THE INDUCED RETICULOCYTE CRISIS OF PERNICIOUS ANEMIA*

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IN A series of 206 cases of pernicious anemia in which remissions were induced by liver therapy we have observed fever, ranging from 102.4° F. to 106° F., coincident with the reticulocyte crisis in 8 cases. In each of these 8 cases a concentrated form of liver extract was administered, to one patient intramuscularly, to the others orally. Every conceivable extraneous cause for fever, such as intercurrent infection and signs of inflammation at the site of injection, was rigidly excluded. In each case the temperature rose rather suddenly, returning to normal more gradually over a period of twenty-four to forty-eight hours. In 5 of the 8 cases reticulocyte counts were being done daily. In 2 of these, the highest temperature coincided exactly with the highest reticulocyte count; in the other 3, it occurred during the period of decline. In the 3 cases in which reticulocyte counts were not being made, the precise relationship between temperature and reticulocytosis was not ascertained, but in each the fever appeared during the time when the crisis was to be expected. The patient whose temperature reached 106° F. had a coincident reticulocytosis of 45 per cent; he was receiving extralin by mouth in doses of 18 capsules per day. In several of the cases, the fever was accompanied by a slight chill; in the others, there were no associated symptoms of any kind. None of the patients had a leucocytosis.

*From the State University of Iowa, Department of Internal Medicine.
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TABLE I

	AGE	SEX	R. B. C. MILLIONS	HB PER CENT	RETICULOCYTES		TEMPERATURE		THERAPY	TEMPERATURE BEFORE THERAPY
					HEIGHT	DAY	HEIGHT	DAY		
S. H.	58	F	2.25	87	----	--	102.4	8	Liver extract	100° R
J. H.	60	M	0.96	20	----	--	103.0	10	Liver extract	Normal
S. H.	56	F	0.70	25	26.8%	7	102.2	9	Liver extract	99°
G. W.	63	M	2.38	65	----	--	105.0	14	Ventriculin	Normal
O. F.	63	M	2.36	63	13.8%	7	102.4	14	Liver extract	100°
J. E.	60	M	1.65	40	20.0%	7	102.0	7	Liver extract I. M.	100°
E. W.	62	M	0.66	18	28.0%	6	105.0	10	Liver extract	101°
O. J.	60	M	0.85	11	45.0%	8	106.0	8	Extralin	100°

There was no apparent relationship between the occurrence of fever and the pretherapeutic erythrocyte level. The continuous low-grade fever characteristic of pernicious anemia during relapse which was manifested by many of these 206 patients disappeared in most cases after the reticulocyte crisis.

We have searched the literature without finding any allusion to fever with reticulocytosis, which in itself is strong evidence that the fever we observed was not referable to the reticulocytosis alone, and we are unable to give a satisfactory explanation of the mechanism. It has been shown that reticulocytes are more fragile than normal erythrocytes and that they do not mature to a significant extent in the circulating blood.[1] The fact that the cholemia is subsiding at the time when the reticulocyte crisis occurs excludes the possibility that increased hemolysis may be the causative factor. Reticulocytes consume more oxygen than mature cells,[2] but inasmuch as there is a gradual decrease in the basal metabolic rate after liver therapy is begun[3] hypermetabolism as a basis for the fever is not tenable. If a foreign protein reaction or an allergic reaction to decomposition products of the erythrocytes or their nuclei is responsible, it is necessary to assume an individual susceptibility in order to account for the infrequent occurrence of the fever.

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THE BLOOD PICTURE IN TWO CASES OF AGRANULOCYTOSIS*

Following Treatment With Neoarsphenamine With Special Reference to Myelocytes and Juveniles

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RECENT literature contains numerous articles on agranulocytosis and like conditions. In but a few instances have there been noted any increase in the myelocytes and juveniles in the circulating blood. Roberts and Kracke,[1] Kerlin,[2] Bethell,[3] Jackson,[4] Schilling.[5] Jackson states: "During convalescence from agranulocytosis there may be, and usually is an outpouring of myelocytes but this stage is soon passed and clinical improvement is evident coincidentally."

It is possible that we have been relying too much on the technician who may, or may not be qualified to make accurate differentiation of the various white blood cells, especially those forms which are not encountered in most daily routine examinations. Or perhaps some qualified individual may become interested, make a few white blood counts and then turn the work over to the technician, thus missing the true picture.

The oxydase reaction in doubtful cases is a valuable aid. The method, as given by Graham,[6] is simple and was used as a check on the Wright's stain in the two cases reported in this paper. During the period where there were no segmented and staff forms present the dfferential count of the granular and nongranular white blood cells was almost identical when the oxydase reaction was compared with slides stained by Wright's method. The myelocytes and juveniles gave a very clear picture with red brown granules in the cytoplasm when stained by the Graham method.

Kracke[7] states: "The term agranulocytosis means an increase in the number of immature granular cells, whereas, granulocytopenia expresses a decrease of granular cells." These two cases developed an increase in the number of immature granular cells. At the same time there was a marked decrease or absence of adult segmented and staff forms.

The following is the history and findings of these two patients. (The medical treatment of the cases for the agranulocytosis was under the supervision of Major H. C. Dooling, Medical Corps, U. S. Army.)

CASE REPORT

Case 1.—(Table I and Chart 1.) An adult white male, aged twenty-seven years, weight 140 pounds, well developed and nourished.

His previous history showed that he was treated for gonorrhea in the early part of October, 1934. On the twenty-ninth of the same month he developed a chancre on his penis, the dark-field being positive for *Treponema pallidum*. Antisyphilitic treatment was begun on

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the same day, consisting of 0.1 gm. bismuth salicylate intramuscularly and 0.6 gm. neoarsphenamine intravenously. One week later a second treatment was given, increasing the neoarsphenamine to 0.9 gm. Two days after the second injection there was noticed a macular eruption scattered diffusely over the entire body. Three days later the eruption was of a deeper color with some itching, and the conjunctiva was congested.

At this time his blood chemistry was within normal limits. The icteric index was 3. The direct and indirect Van den Bergh tests were negative. The white blood count was 5,600; differential 61 per cent segmented, 4 per cent staff, 8 per cent juvenile, 6 per cent eosinophiles, 12 per cent lymphocytes, and 9 per cent monocytes.

By November 12, the patient being apparently well, it was decided to continue the antisyphilitic therapy. Bismuth 0.1 gm. and neoarsphenamine 0.5 gm. were given. There was no evidence of any reaction and seven days later the same treatment was repeated. This made a total of 1 gm. neoarsphenamine in eight days.

Five days after the last treatment he was readmitted to the hospital with a diagnosis of external hemorrhoids. He had been doing duty for the past twelve days.

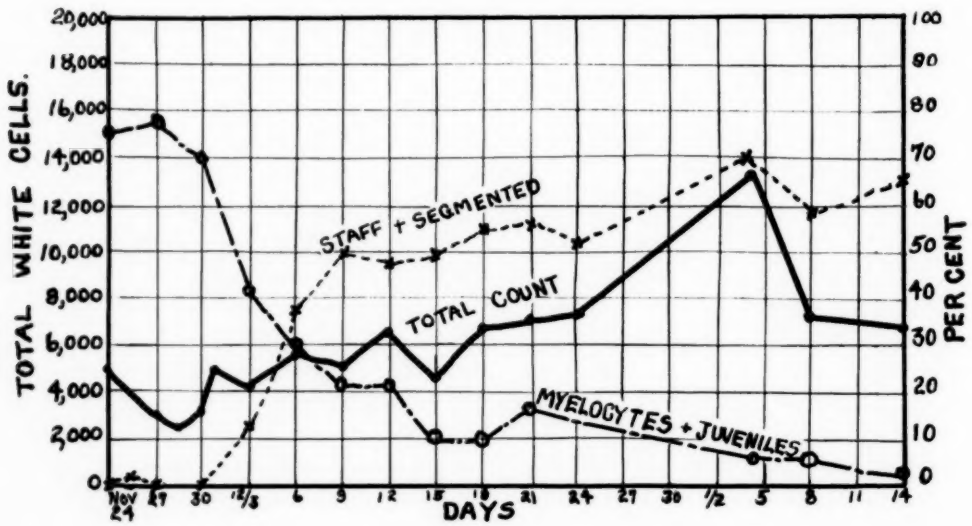

Chart 1.

Physical examination on admission was essentially negative, except for a slight reddening of the throat and some hypertrophy of the tonsils. There were no ulcerations on the tonsils or gums. There were three small external hemorrhoids present.

On admission the temperature was 97.4° F., pulse 128, and respiration 20. At 8 P.M. the temperature was 102° F. The following day he complained of marked weakness and seemed acutely ill. His temperature was 104°. Upon reexamination, the only positive findings were the congested pharynx and the external hemorrhoids. A blood count revealed a total of 4,900 white cells. There were no segmented cells present, but there were a considerable number of myelocytes and juveniles. The patient was transferred to the Medical Service with a diagnosis of agranulocytosis following antisyphilitic treatment with neoarsphenamine.

The treatment consisted of nucleotide (K-96) and glucose intravenously. The nucleotide was given for eight consecutive days. Liver extract was started on the second day, both intramuscularly and orally. He continued to have a high temperature for nine days. During this time he seemed prostrated and the gums and mucous membrane of the pharynx became markedly ulcerated. The breath was very foul. The hemorrhoids ulcerated and disappeared as if they had been burned off with a cautery.

The patch test with neoarsphenamine was negative on two different days. The fragility test revealed a slight increase in the resistance of the red blood cells, hemolysis beginning in 0.42 and complete in 0.30 per cent saline.

The clinical course closely paralleled the change in the blood picture, the patient improving markedly as the segmented and staff forms returned to the circulating blood. This case was more severe than Case 2 and it is interesting to note that there were a greater number of myelocytes and juveniles and less eosinophiles and basophiles than in the second case. Also he did not show as marked an increase in the segmented forms during recovery as Case 2. It was also noted that the staff forms appeared before the segmented. There was only one staff and no segmented forms noted during the first seven days.

TABLE I

CASE 1. TOTAL WHITE BLOOD CELLS AND DIFFERENTIAL COUNT

DATE	TOTAL COUNT	BASO-PHILES	EOSINO-PHILES	MYELO-CYTES	JUVE-NILE	STAFF	SEG-MENTED	LYMPH-OCYTES	MONO-CYTES	MISCEL-LANE-OUS
11/24/34	4,900		2	55	21			15	5	1
25	4,200			52	26	1		18	3	
26	3,500	1		47	25			14	13	
27	3,100			72	5			16	7	
28	2,500		1	53	14			24	4	4
29	2,950	1	1	31	29			33	5	
30	3,000	2	2	34	36			20	6	
12/ 1/34	4,700	1	4	23	29	2	1	37	3	
2	4,350	1	6	16	32	9	1	29	6	
3	4,100	1	11	17	24	9	4	31	3	
4	5,800		4	17	22	13	8	28	8	
5	5,700	1	1	21	15	14	24	22	2	
7	6,500			6	23	9	33	29	1	
9	5,500		2	7	15	10	40	24	2	
11	5,300		2	5	12	11	37	25	8	
12	6,400		3	9	12	5	42	24	5	
13	7,400	1	5	4	12	2	47	25	4	
14	6,550	1	2	3	9	3	38	39	6	
15	4,550		5	2	8	3	45	30	7	
17	5,350		2	6	9	5	49	22	7	
19	6,600		3		10	4	52	25	6	
22	6,950	1	6		17	4	46	25	1	
26	7,100		4		9	1	50	32	4	
1/ 4/35	12,750		1		6	4	66	22	1	
8	7,350	1	4		6	4	55	28	2	
15	7,000			1	4	5	67	20	3	
19	7,800			1	2		65	29	2	
22	8,600		3	1	7	9	61	11	8	
2/14/35	9,950	1	2		6	3	49	34	5	

In the graphic Chart "1" the myelocytes and juveniles and the staff and segmented are grouped together. The clinical course of the patient corresponded with this grouping and as the staff and segmented increased and the myelocytes and juveniles decreased, the condition of the patient improved accordingly.

CASE 2.—(Table II and Chart 2.) Patient 2 was a white male, aged nineteen, weight 160, a soldier, well developed and nourished. He had bronchopneumonia ten years ago and the usual diseases of childhood. He contracted gonorrhea in April, 1934, and was admitted to the hospital in October, 1934, for chronic urethritis. It was then found that his Wassermann and Kahn tests, repeatedly, were strongly positive. He was therefore started on antisyphilitic treatment as follows: Oct. 14, 1934, 0.1 gm. bismuth salicylate and 0.5 gm. of neoarsphenamine. October 21, the same amount of bismuth with 0.9 gm. of neoarsphenamine. November 3 this was again repeated. This made a total of 2.3 gm. of neoarsphenamine over a period of twenty-one days. There were no reactions following the first two treatments. However, from the time of the last treatment he had been feeling weak, having a mild sore throat. He had

been doing his regular duty, consisting of the rather strenuous cavalry drill. On the morning of November 9, while at drill, this being six days after his last intravenous neoarsphenamine, he became dizzy and dismounted from his horse. He had a few chills, vomited, and then developed a severe headache, together with a pain in his chest.

On admission to the hospital he was acutely ill. His skin was flushed, his respirations were 30, temperature 104° F., and pulse 120, being full and regular. He was semilethargic, and could be aroused with difficulty to answer questions. He was slightly irrational when aroused. There was a foul odor to his breath and his throat was red. The gums were of normal appearance. The tonsils were moderately inflamed, but there were no ulcers present. No enlarged glands were noted. The heart sounds were clear, and there were no murmurs present. There was a slight dullness at the right base with decreased breath sounds in this region. The abdomen was scaphoid. The liver and spleen were not palpable. He was therefore put under observation for pneumonia.

The x-ray revealed only a residual of an old pneumonia, and compared with the films taken on his release, two years ago, there were less markings. There was no evidence of any acute process in the lungs. However, a blood count was made and the total white count was

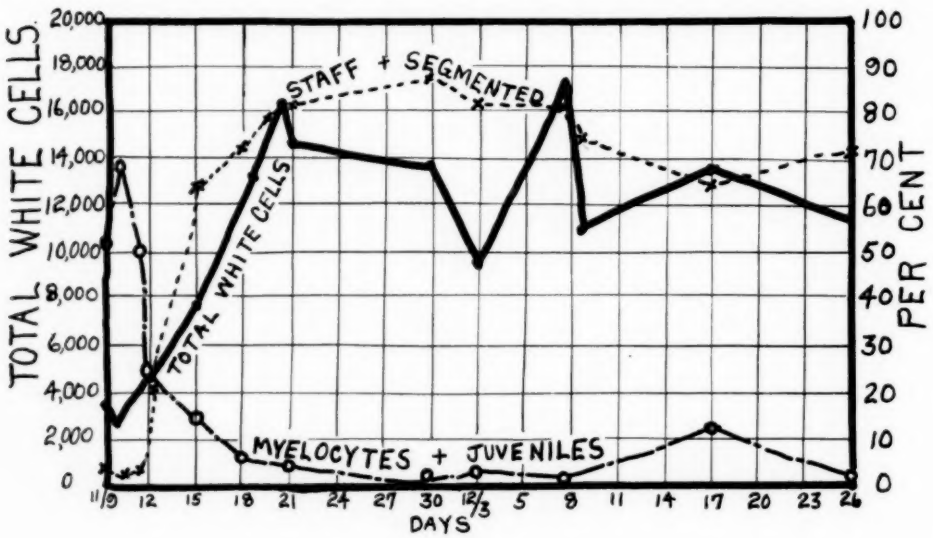

Chart 2.

3,800 with only two segmented cells per hundred noted. There were a considerable number of juveniles and myelocytes present.

The next day, November 10, his condition was grave. His throat was red, yet there were no ulcerations on the gums or tonsils. He was given 10 c.c. nucleotide (K-96) intramuscularly. He was nauseated during the entire day and could not retain his food. Thirty cubic centimeters of 50 per cent glucose was given intravenously. On this day, not a single segmented cell could be found on the slide. The total count was 2,700 and 10 myelocytes and 57 juveniles per 100 were noted.

The following day, November 11, the same treatment was repeated with the addition of an antiseptic gargle and mouth wash, as he was developing a slight ulceration on the gum margin. He was retaining some food. One segmented per hundred white cells was noted.

The fourth day, November 12, the same treatment was continued with the addition of liver. The ulcerations on the gums had increased. The temperature which had been 104° on the first two days was now 102.2° and there appeared 13 per cent segmented forms in the blood smear. From this time on he made a rapid recovery, the improvement paralleled the increase in the segmented and staff forms in his blood smears.

The temperature continued to show a slight afternoon elevation during his stay in the hospital.

Other laboratory findings were: throat culture positive for hemolytic streptococci and negative for Vincent's organisms during the acute stage. A blood culture taken November 10 was negative. The icteric index November 13 was 3.75. A patch test with neoarsphenamine was markedly positive November 13. However, this test repeated after his recovery, January 20, was negative. The urine was within the normal limits. The fragility test showed a slight increased fragility of the red blood cells, hemolysis beginning in 0.48 and complete in 0.34 per cent saline. Many different bacteria were found in smears taken from the ulcers during the period November 10 to 12, yet no white blood cells were present.

Table II shows the progress of the blood count with special reference to the differential white cell count. It shows a marked shift to the left (Schilling index) with a later shift to the right as the patient improved. During convalescence there was a marked hyperleucocytosis involving the segmented, staff, and juvenile forms, the highest count being about eight days after his first signs of improvement.

Chart 2 gives a grouping of the segmented and staff and the myelocytes together, charted with the total white cell count.

TABLE II

CASE 2

NUCLEOTIDE	TEMPERATURE	DATE	TOTAL COUNT	BASOPHILES	EOSINOPHILES	MYELOCYTES	JUVENILE	STAFF	SEGMENTED	LYMPHOCYTES	MONOCYTES	MISCELLANEOUS
10 c.c.	104.0	11/ 9	3,800	4	2	8	43	3	2	19	17	2
10 c.c.	104.0	11/10	2,700	1	1	10	57	3		16	5	5
10 c.c.	102.4	11/11	4,100			6	45	3	1	21	18	5
10 c.c.	102.2	11/12	4,600	1	2	2	22	8	13	34	13	5
	101.0	11/13	5,250		8		8	18	28	31	3	4
	99.4	11/14	6,400			1	6	13	57	18	5	
	99.6	11/15	7,800		3	1	13	12	52	14	3	2
	99.6	11/16	10,600		2	1	6	10	61	20		
	99.0	11/19	13,400			1	5	6	68	18	2	
	98.6	11/20	16,150	1			5	6	71	11	6	
	99.0	11/21	14,500	1			5	9	72	9	3	
	98.0	11/30	13,800			2	1	4	83	9	1	
	99.0	12/ 3	9,400				3	1	82	12	2	
	99.0	12/ 7	17,200				2	4	77	9	8	
	98.0	12/ 9	9,200			1	3	4	70	19	3	
	99.0	12/14	11,600			2	7	2	73	9	7	
	99.8	12/17	13,600		1	3	10	4	61	19	2	
	99.0	12/26	9,950		2		9	1	71	12	5	
	98.0	1/ 4	6,500		4		3	6	66	14	7	
	99.0	1/ 9	9,400		4		5	1	61	26	4	
	99.0	2/16	14,400	1	2		2	1	80	11	3	

NOTE: Under miscellaneous were noted lymphoblasts and basket cells. Blood platelets decreased in number about one-half for first four days, platelets being markedly increased in size, many as large as red blood cells. From November 13 on, the platelets were markedly increased in number and showed marked variations in size.

The red blood count was normal throughout, being from 4,500,000 to 5,000,000. The eosinophiles and bashophiles present during the first five days were of the juvenile type.

COMMENT

It will be noted that these patients came to the hospital without ulcerative lesions and developed them later. One patient was admitted with external hemorrhoids that ulcerated and completely sloughed about the sixth day after admission. Therefore, it seems probable that the ulcers on the

mucous membranes, in those locations where there are normally pathogenic bacteria in close contact with the mucous membranes, namely, the gums, tonsils, and anus, were due to the lack of segmented and staff forms (phagocytes) in the circulating blood. Although there were a considerable number of myelocytes and juveniles present, nevertheless, the ulcerations started and progressed. When the segmented and staff forms returned to the circulation, the patients improved rapidly, and simultaneously the ulcerations began to heal. This, together with the fact, that both of these patients had received neoarsphenamine (which had its effect on the bone marrow, causing a loss of the phagocytes), would tend to rule out the theory that the toxic products of the bacteria are responsible for the changes in the bone marrow.

When we consider the great number of patients who are treated with neoarsphenamine without any reaction and then suddenly one is admitted with agranulocytosis following administration of small amounts of this drug, there is probably one explanation and that is on the basis of allergy. Case 2 received only three intravenous injections. Case 1 received two and was permitted to delay his treatment because of a reaction and then after two more medium-sized injections, he developed a severe reaction. This is in contrast with the cases of arsenical dermatitis and arsenical jaundice which usually follow more prolonged treatment. It is generally accepted that the benzene ring of the neoarsphenamine is the disturbing factor in agranulocytosis. This same benzene ring forms a part of many drugs recently reported affecting the bone marrow.

It is also noted that the myelocytes were found on the first blood count, that is, before the nucleotide was given. Therefore, we cannot attribute the outpouring of the myelocytes and juveniles to the action of the nucleotide.

Another interesting observation is that the portion of the bone marrow which has to do with the production of the blood platelets must also have been affected and that part having to do with the formation of the red blood cells seemed not to have been disturbed. The blood platelets in both cases were reduced in numbers, and there were many very large ones present during the period when the segmented and staff forms were lacking. When the segmented and staff forms returned, there was a concomitant marked increase in the number of platelets with many large ones present.

In view of the marked improvement in the patients upon the return of the staff and segmented forms, these are grouped together. The myelocytes and juveniles are grouped together because they seem to have no phagocytic activities. That period where the segmented and staff forms increased in number and the myelocytes and juveniles decreased corresponds with the period of marked improvement in the condition of the patients.

It is also interesting to note the changes in the daily blood picture of Case 1. (He was the more severe of the two from the clinical standpoint.) First 55 per cent myelocytes and 21 juveniles with no staff or segmented. Seven days later the juveniles were more abundant than the myelocytes. Two days later there were 9 staff and 1 segmented. Then finally the segmented were in the majority. This demonstrates the value of the Schilling count, and is in accordance with the development of these cells.

There are cases where it seems very probable that the toxic products of bacteria may cause dysfunction of the bone marrow. However, one must consider that the bone marrow may be abnormal from the start from various causes. Perhaps allergy in the form of sensitivity to certain drugs may play an important part.

The following case is an example:

A white male, aged sixty-two years, was admitted to the hospital two days after going to a chiropodist who pared a corn from his foot. When admitted he was seriously ill. His temperature was 105° F. and his pulse rapid. The blood counts revealed 500 to 1,000 white blood cells, all of which were small lymphocytes. A blood culture was positive for hemolytic streptococci. He died four days after the chiropodist attended him. Death in this case was probably due to septicemia, as four days with no phagocytes present in the blood stream and no other complications would be the exception. However, a poor functioning bone marrow may have been the basic cause.

A person who patronized the chiropodist might also patronize the corner drug store and use drugs that might be dangerous. Certain drugs are known to affect the bone marrow and with a loss of the phagocytes, bacteria may invade any part of the body. The history as to the use of drugs was not obtained in this case as little was known at that time with regard to these harmful drugs. Now that it is a well-established fact, an attempt to elicit the history of drug administration should be made in these cases.

Patients 1 and 2 were probably allergic to neoarsphenamine. One gave a marked positive patch test. The other developed an allergic type of reaction.

Another patient was observed by me two years previously. He was sensitive to neoarsphenamine and developed a reaction every time it was given. It was six months before his blood picture returned to normal. He was also sensitive to quinine. This patient was given quinine to determine what effect it might have on his blood picture. Roth[8] states that quinine causes a pronounced leucocytosis; at first there is an increase in the number of lymphocytes which he believes is due to contraction of the spleen and lymphatic tissue, but later there is an increase in the number of polymorphonuclear cells. Quinine in this case, in doses of 5 gr. three times daily, was given over a period of six days. There was an increase in the segmented cells during this period. However, as this man was sensitive to quinine as well as neoarsphenamine, this treatment was discontinued.

CONCLUSIONS

Two cases are presented, following treatment with neoarsphenamine, with an increase in the circulating myelocytes and juveniles and a decrease in the number of segmented and (stab) staff forms with a marked shift to the left in the Schilling index.

1. As these patients were admitted with no ulcerative lesions and later there appeared a disintegration of the tissue where pathogenic bacteria are in intimate contact with the mucous membranes, namely, the gums, tonsils, and anus; it is presumed that these lesions were due to the absence of the phagocytes (the segmented and staff forms) from the circulating blood.

2. Smears taken from the lesions revealed only bacteria and débris, there being no white blood cells present. Therefore, the bacteria flourished during the absence of the phagocytes.

3. Myelocytes and juveniles apparently have no protective value in combating local infections, there being a considerable number of these forms in the circulating blood before and during the time that the ulcerations appeared and progressed.

4. The segmented and staff forms, by means of their flexibility, may pass through the capillary walls and act as phagocytes, whereas, the myelocytes and juveniles with their large single nuclei, were unable to pass between the intercellular spaces.

5. From the standpoint of the clinician, the granulocyte count made according to Schilling, might be divided into two main groups, i.e., those cells possessing phagocytic powers (staff and segmented), and those having little if any phagocytic powers (myelocytes and juveniles).

6. With this grouping it was noted that the clinical picture of the patient corresponded with the charts. That is, there was noted a marked improvement in the patient when the staff and segmented forms appeared in the circulating blood and the myelocytes and juveniles decreased in numbers.

7. It is not probable that the nucleotide was the factor causing the myelocytes and juveniles to appear in the circulating blood, as these forms were present in increased numbers before the nucleotide was given.

8. The neoarsphenamine was the cause of the condition by its effect on the bone marrow.

9. These patients were probably allergic to neoarsphenamine.

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STUDIES ON THE CIRCULATION: THE DYE INJECTION METHOD*

The Effect of Digitalis Upon Patients With Normal Cardiovascular Systems

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DIGITALIS has been known for many years as the sovereign remedy for heart disease. The mechanism of its action on the circulatory system in health and disease has been the subject of numerous investigations. In simple perfusion experiments the effect of digitalis upon the isolated frog or mammalian heart is that the organ empties itself more completely. In the more intricate experiments as the heart-lung preparation in the dog, Cohn and Steele[1] have shown that the administration of therapeutic doses of digitalis to the failing and dilated heart causes an increase in minute output, whereas in the case of the healthy heart, normal in size, the opposite is true. They felt that the effect on the tone of the heart is the outstanding phenomenon. They refer to tone as meaning the diastolic volume of the heart, the length of the fibers in a given case or the fitness of the muscle fibers.

Abundant evidence has been forthcoming in recent years to show that the whole story of the action of digitalis in the body cannot be adduced from experiments upon isolated organs, but rather upon the interpretation of the changes that occur in the intact organism after readjustments have taken place in the body as a whole.

In the normal dog, Harrison and Leonard,[2] Stewart and Cohn,[3] and Dock and Tainter[4] found a decrease in output following the administration of digitalis. Stewart and Cohn are of the opinion that the drug acts primarily upon the heart and that the decrease in output is the consequence of the heart becoming small, whereas Dock and Tainter express the opinion that the decrease in output is the result of the constricting action of the drug upon the hepatic veins. They believe that in the dog, the constriction is at the caval openings of the veins, whereas in man and cat, the constriction occurs in the hepatic vein radicals. As proof of their contention they cite experiments which show a fall in right auricular pressure and a simultaneous rise in portal vein pressure and liver engorgement following the administration of digitalis.

It would appear, therefore, in the light of the above conclusions, that the pharmacologic action of digitalis in the body of the dog is still open to further investigation.

In the normal human being the studies of the action of digitalis have been limited principally to the cardiac size and output, venous pressure, velocity of blood flow and total blood volume. Following the administration of this drug, Burwell and others,[5] using the carbon dioxide method, and

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Stewart and Cohn,[3] using the acetylene method, reported a decrease in output. Stewart and Cohn[3] report the size of the heart to be decreased. They found no significant changes in venous pressure, whereas Rytand[6] found a decrease; Blumgart[7] found no substantial change in the velocity of blood flow and Ceccarelli,[8] using the fluoroscene method as advised by Wollheim, came to the same conclusion. Groscurth and Bansi[9] are of the opinion that digitalis brings about "a useful sort of help" in healthy persons doing hard work. They feel that this is accomplished mainly through the slowing of the blood stream. They interpret the slowing of the stream "alone in consequence of the widening of the capillary cross-section and therewith the increase of the exchange surface and the increase of time for use of this exchange surface." Wollheim[10] observed a decrease of 5 to 18 per cent in blood volume even in cases in which the pulse remained unchanged. It should be noted that the blood volume figures of Wollheim were obtained twenty minutes after the injection of 2 c.c. of digitalis.

With the above as a brief background of the results of human and animal experimentation concerning the effect of digitalis, we wish to present our study on hemodynamics based on the use of the dye-injection output method.

Dye-Injection Method.[11-13]—With the patient in the recumbent position, 4 c.c. of an aqueous solution containing 300 mg. of brilliant vital red is injected quickly into the vein of the upraised arm. The femoral artery is punctured with a conveniently shaped needle and continuous samples of blood are collected in glass tubes held in place about the drum of a kymograph. A time marker records the speed of the drum, thus making it possible to determine the time each tube occupies during the test. Standards are made by adding the dye solution to the patient's blood. The concentration of the dye in milligrams per liter is determined in each sampling tube.

The time-concentration curve is obtained by plotting the coordinates on a semi-logarithmic scale, where the time (absissae) is linear and the concentration (ordinates) is logarithmic. This procedure makes the descending limb of the curve a straight line up to the time when recirculation begins, and by prolonging this line we can determine how much of the dye is on its first circulation.

By this method it is possible to obtain: (1) The velocity of blood flow between needles, (2) the flow per minute, (3) the volume of actively circulating blood between needles, (4) the total volume of actively circulating blood in the body, and (5) the stroke volume.

Venous Pressure.—The direct venipuncture method was used following the technic recommended by Rytand.[6]

Heart Size.—The patient was in the recumbent position. The x-ray tube was fixed 7 feet from the patient. From two to two and one-half seconds' exposure was used. The heart area was determined by the use of the planimeter.

Specific Gravity of Plasma and Serum.—The drop method of Barbour and Hamilton[14] was used. For convenience heparinized venous blood was used for plasma and arterial blood for serum specific gravity determinations.

Cell Volume.—Eight cubic centimeters of heparinized venous blood was placed in each of two 15 c.c. graduated centrifuge tubes (the blood used for standards in the dye method) and centrifuged rapidly for one hour or until the cells were well packed.

Procedure.—All of the subjects were in the postabsorptive state in the morning. As the actual time of securing the femoral blood samples for the output determinations was less than one minute, it was possible with the patient in the recumbent position and a suitably placed x-ray tube to obtain much of our data in a minute's time. The venous and arterial pressures were taken just before and the blood volume samples three minutes following the begin-

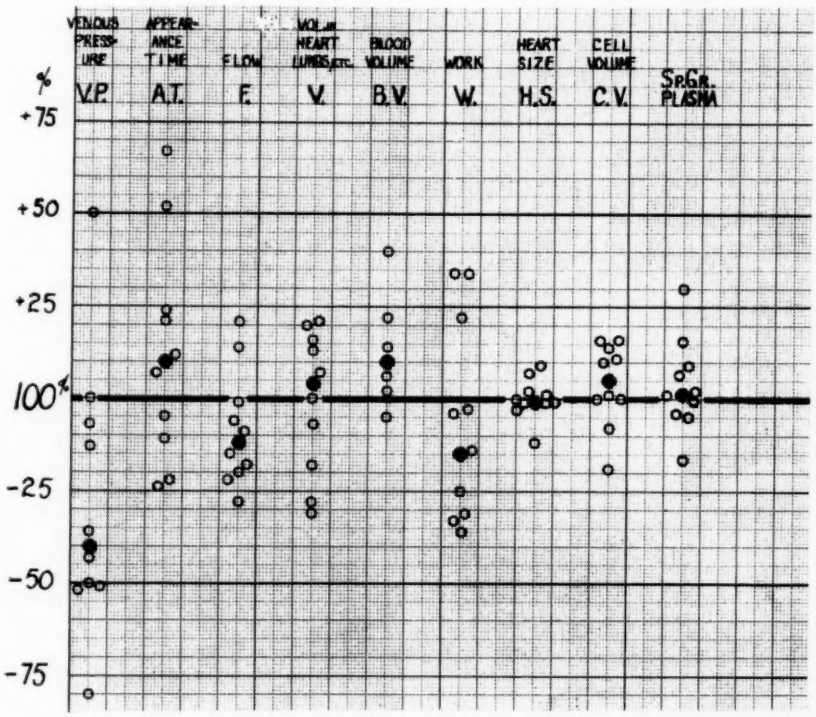

Fig. 1.—Showing the median values of the various factors in percentage of initial normal, together with the distribution of the individual cases. V.P., Venous Pressure. A. T., Rate of flow between antecubital vein and femoral artery. Flow, Output per minute. V., Volume of circulating blood between point of injection (antecubital vein) and point of sampling (femoral artery). B. V., Total circulating blood volume. W, Flow times mean blood pressure. H. S., Cardiac silhouette area. C. V., Cell volume. Sp. gr. Pl., Specific gravity of plasma.

ning of the output determination. Once the test was begun, the patient at no time was changed from the recumbent position.

Two days following a successful output determination (the time required for the dye to be excreted) one to one and two-tenths grams of digitalis in tablet form (digitora) was given during the space of three hours, and twenty-four hours later the second determination was carried out. It has been shown by Stewart and Cohn[3] and Rytand[6] that twenty-four hours are usually required to obtain the maximum effects.

Results.—Table I shows a summary of the findings. With the exception of the pulse and the blood pressure each value in every case has a column showing the percentage of the initial normal, and Fig. 1 is constructed to show the median, as well as the case distribution of each factor in terms of percentage of the initial normal. It will be seen in glancing at Fig. 1 that some of the factors show a rather decided deviation from the normal median values; at the same time the scatter of the individual values is quite as prominent.

Effect on Venous Pressure.—The median venous pressure of the group following digitalis therapy is decidedly decreased, being 61 per cent of normal. The changes range from 20 to 150 per cent of the initial normal.

The cases which show no change or a decrease in venous pressure likewise show no change or a decrease in the flow. Case 130 is an exception. Case 161 shows a marked increase in venous pressure, as well as a marked increase in

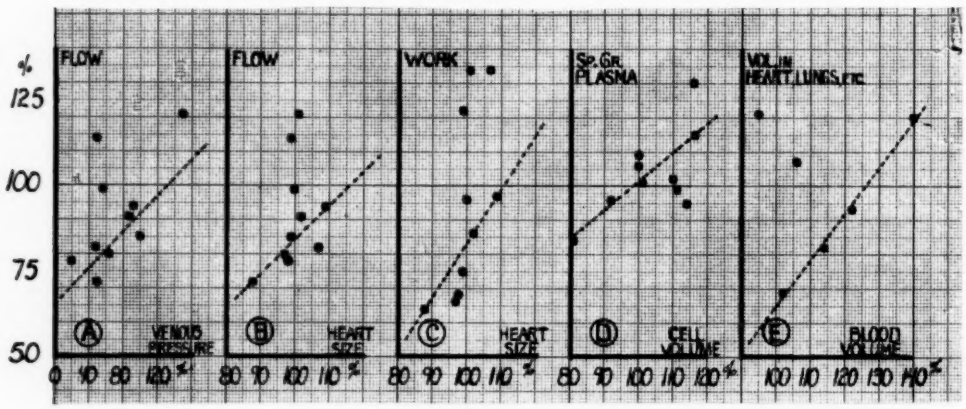

Fig. 2.—Showing the interrelationship of the various factors in terms of per cent of initial normal. Flow, Output per minute. Work, Flow per minute times mean blood pressure. Heart size, Cardiac silhouette area. Sp. gr. Pl., Specific gravity of plasma. Vol. of blood in heart and lungs, Volume of circulating blood between point of injection (antecubital vein) and point of sampling (femoral artery).

flow. However, in the individual cases of the groups when the venous pressure and flow are plotted against each other there exists a close linear relationship in not more than 70 per cent of the cases (Fig. 2 A).

There is no correlation between venous pressure and the other factors studied, namely, the velocity of blood flow, the stroke volume, the amount of blood in heart and lungs, the total blood volume, the heart area, the specific gravity of the plasma, or the work.

Velocity of Blood Flow.—While there is a tendency toward a decrease in the velocity of blood flow, nevertheless the changes are not substantial, and there is no correlation with the other factors.

Flow.—The median flow is 88 per cent of initial normal. The deviations are from 72 to 121 per cent of initial normal.

In 80 per cent of the cases there is a definite linear relationship between flow and heart area (Fig. 2 B).

TABLE I*

TWENTY-FOUR-HOUR EFFECT OF DIGITALIS (1 TO 1.2 GM.) IN THE HUMAN WITH NORMAL CARDIOVASCULAR SYSTEM

CASE	AGE	PULSE	B. P.	V. P.	% INITIAL NORMAL	A. T.	% INITIAL NORMAL	FLOW/SA	% INITIAL NORMAL	V/SA	% INITIAL NORMAL	BV/SA	% INITIAL NORMAL	WORK/SA	% INITIAL NORMAL	HEART SIZE	% INITIAL NORMAL	CELL VOL.	% INITIAL NORMAL	SP. GR. PLASMA	% INITIAL NORMAL
165	23	72	110/70	70		9.0		3.48		1.19		2.58		4265		99		52.0		P-1.0268	
♂		60	110/75	65	93	10.9	121	3.28	94	1.28	107	2.75	106	4150	97	108	109	52.0	100	P-1.0279	109.0
161	19	87	124/80	40		9.2		4.23		1.35		2.41		5875		104		44.9		P-1.0284	
♂		66	140/84	60	150	7.0	76	5.14	121	1.11	82	2.75	114	7830	134	105	101	45.5	101	P-1.0261	100.5
164	18	72	120/75	105		14.8		3.81		1.97		2.53		5080		112		45.5		P-1.0248	
♂		66	110/80	60	57	13.2	89	3.77	99	1.37	69	2.59	102	4875	96	112	100	52.6	116	P-1.0273	130.0
120	27	81	116/70	83		9.2		3.32		1.35				2622		106		38.5		P-1.0228	
♀		78	120/70	40	48	14.0	152	2.72	82	1.56	116			3513	134	113	107	38.5	100	P-1.0279	106.4
130	39	71	110/62	82		13.1		3.88		1.53		1.89		4538		136		45.5		P-1.0268	
♂		62	120/65	40	49	12.5	95	4.43	114	1.83	120	2.65	140	5540	122	135	99	50.7	116	P-1.0225	115.3
131	45	67	160/80	30		26.0		4.00		3.43				6530		124		35.0		P-1.0243	
♂		60	140/74	-15	50	20.4	78	2.89	72	2.45	72	2.79		4200	64	109	88	39.0	111	P-1.0266	99.4
133	30	81	128/80	55		7.6		5.47		1.33				7740		120		47.4		P-1.0227	
♂		88	106/70	35	64	12.3	162	4.36	80	1.50	113			5220	67	117	97	52.0	110	P-1.0202	102.0
145	29	75	115/84	75		12.5		2.72		1.20		2.18		3670		123		49.3		P-1.0263	
♂		87	100/75	15	20	15.5	124	2.12	78	1.19	100			2540	69	120	98	56.3	114	P-1.0256	95.0
142	49	83	125/90	30		13.6		3.90		1.48		2.36		5680		112		46.8		P-1.0272	
♂		63	110/80	30	100	14.6	107	3.30	85	1.38	93	2.89	122	4263	75	111	99	37.7	81	P-1.0235	83.6
134	38	88	128/95	80		7.5		3.65		0.78		2.05		5510		90		48.0		P-1.0271	
♂		69	124/85	70	87	8.4	112	3.33	91	0.94	121	1.95	95	4712	86	92	102	44.2	92	P-1.0270	96.1

*V. P., Venous pressure. A. T., rate of flow between antecubital vein and femoral artery. Flow, output per minute. V, volume of circulating blood between point of injection (antecubital vein) and point of sampling (femoral artery). B. V., total circulating blood volume. S. A., surface area. Work, flow per minute times mean blood pressure. Heart Size, cardiac silhouette area. Sp. Gr. Pl., specific gravity of plasma.

Effect on Stroke Volume.—The median stroke volume is increased to 110 per cent of initial normal. The deviations are from 67 to 159 per cent of initial normal. There is no correlation with the other factors.

Total Blood Volume.—Only five cases were determined in this series. There is a tendency for the total circulating blood volume to be increased. This is not in keeping with the findings reported by Wollheim; nevertheless, the conditions are hardly comparable, in that he injected the digitalis and made his determinations soon after the injection (twenty minutes), whereas with us the drug was given by mouth, and the determination was made twenty-four hours later.

In four of the cases there is a striking linear relationship between the total blood volume and the volume in the heart and lungs (the circulating blood between the point of injection [antecubital vein] and the corresponding vessels to the point of sampling [femoral artery]). The latter we have termed V (Fig. 2 E). The V in four of these cases is decreased in about the same proportion as the circulating blood volume in the greater circulation is increased. It is evident that the engorgement is either in the extremities or in the splanchnic area.

Heart Area and Work.—The percentage of deviation from initial normal in each of these two factors has a scatter which causes the median value of the heart area to fall near the initial normal, whereas the median for work falls well below. However, when the values are plotted against each other, we find that seven out of the ten cases show some linear correlation (Fig. 2 C). There is no correlation of the other factors with work.

Cell Volume and Specific Gravity of the Plasma.—The median values of these two factors show little or no change following the administration of digitalis (Fig. 1). The scatter of the individual cases in the cell volume is from 81 to 116 per cent of initial normal, and in the specific gravity of the plasma from 84 to 116 per cent of initial normal. There is some correlation between the cell volume and the specific gravity of the plasma (Fig. 2 D). No such correlation exists with the other factors studied.

COMMENT

In reviewing the literature of the action of digitalis on the human being with normal cardiovascular system, one is impressed with the decided divergence of opinion by some of the most outstanding workers in this field. We find that one school of thought stresses the action of the drug upon the heart, whereas another school emphasizes its action upon the peripheral circulation. The methods we have used in the study of hemodynamics have the advantage of determining almost simultaneously without change in the position of the patient, the venous pressure, vital capacity, velocity of blood flow, flow, total blood volume, etc.

Our results show that twenty-four hours after the oral administration of fairly large doses of digitalis, the heart area may increase, decrease or remain unchanged. The flow on the whole is diminished, though some cases show an increase and others are unchanged. There is, however, some correlation be-

tween heart area and flow. The total circulating blood volume, as well as the volume in heart and lungs (V), has a tendency to be increased and in both there is a fairly wide spread in values. The significant finding, however, is the striking linear relationship that some of the cases show between these two factors. In four out of six cases the change in total circulating blood volume exceeds the change in blood in the heart and lungs (V) by 20 to 30 per cent. These findings seem to prove that in some cases at least the capillary cross-section in the greater circulation is increased over that of the lesser circulation, and tend to uphold the contention of Dock and Tainter that the engorgement takes place in the liver or splanchnic vessels.

SUMMARY

1. In ten cases with apparently normal cardiovascular systems, the hemodynamics was studied before and twenty-four hours after the oral administration of from one to one and two-tenths grams of digitalis (digitora tablets).

2. By the use of the dye-injection output method, it was possible to determine simultaneously the velocity of blood flow, the flow per minute, the total circulating blood volume, the volume of circulating blood in the lungs and heart (V), the cell volume and the specific gravity of the plasma. It was possible also to obtain almost at the same time and certainly without any change in position of the patient, the venous and arterial blood pressures, the vital capacity and the cardiac silhouette area.

3. In all of the factors studied, it was found that digitalis may cause an increase, a decrease, or no change at all in the individual values. The median of any group may show a decided trend; nevertheless, the spread about the initial normal was prominent. This was particularly true of the venous pressure and flow per minute. In the former the median value was 60 per cent of the initial normal, whereas one case was 150 per cent of the initial normal; in the latter, the median value was 88 per cent of the initial normal, whereas one case was 121 per cent of the initial normal. When the venous pressure and the flow per minute are plotted against each other there exists a fairly close linear relationship in about 70 per cent of the cases.

4. There was evidence of some correlation between work and cardiac silhouette area, flow per minute and cardiac silhouette area, specific gravity of the plasma and the cell volume, and total circulating blood volume and the circulating blood volume in the lungs and heart. We believe the latter correlation is significant, in that it shows that digitalis in some cases exerts a peripheral action to cause an increase of circulating blood in the greater circulation, though not necessarily associated with changes one way or the other in the flow per minute, venous pressure or velocity of blood flow.

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THE RELATIONSHIP OF VITAMIN C TO THE HEMORRHAGIC DIATHESES*

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THE isolation and preparation of vitamin C in pure form has stimulated interest in the antiscorbutic vitamin in many fields. The striking response of the hemorrhagic manifestations of scurvy to vitamin C therapy has led to the use of cevitamic acid in other types of hemorrhage. Favorable therapeutic effects of the intravenous administration of cevitamic acid have been reported in several types of capillary bleeding, including that due to hemophilia, thrombocytopenic purpura, Schönlein's purpura and metrorrhagia.[1-3] Our own experience in a series of individuals with capillary bleeding of various types has been difficult to evaluate. In many patients bleeding continued unabated after the intravenous administration of 100 mg. of cevitamic acid. In others hemorrhage ceased within a few hours after the injection. In clinical observations of this type the *post hoc ergo propter hoc* argument is notoriously unreliable unless fortified by confirmatory evidence.

In the present study, clinical observations of hemorrhagic phenomena have been correlated with blood changes, capillary resistance, and the urinary excretion of vitamin C before and during the prolonged administration of large amounts of orange juice.

Harris and Ray[4] have shown that the administration of large test doses of vitamin C to normal individuals results in a marked increase in the urinary excretion curve if the diet has been adequate. In the "unsaturated," scorbutic, or semiscorbutic subject, however, the urinary excretion remains low until saturation has taken place. In this investigation, the excretion of a large proportion of ingested vitamin C has been assumed to indicate saturation of the tissues with antiscorbutic material.

Four patients with hemophilia and two patients with atypical chronic thrombocytopenic purpura were studied. After preliminary clinical and laboratory observations, each patient was given, in addition to the regular diet, a daily ration of orange juice equivalent to 100 or 200 mg. of vitamin C. With one exception (C. M.), the usual diet had contained an average amount of antiscorbutic foods. Repeated observations of capillary resistance, twenty-four-hour excretion of vitamin C, and blood studies were made during the experimental period. Estimations of cevitamic acid in the urine were made by titration with a standardized solution of 2:6-dichlorophenolindophenol according to a method described by Harris and Ray.[4] Capillary resistance was determined with a modification of the apparatus described by Dalldorf.[5] The cup was applied to the inner surface of the upper arm for one minute; results

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were expressed as the amount of negative pressure necessary to produce macroscopic petechiae. The method of Lee and White was used in determining coagulation time. Bleeding time was determined by the method of Duke.

CASE REPORTS

CASE 1.—J. K., a twenty-year-old youth, had had repeated frequent hemorrhages into joints, muscles, skin, and mucous membranes, and episodes of hematuria since birth. Both he and his brother, W. K., who also has hemophilia, have been studied and reported by Birch[6] with reference to the effect of ovarian preparations. Neither has had any glandular therapy since 1932.

The patient was admitted to the hospital on May 4, 1935, because of an acute hemarthrosis of the left knee and massive hematoma of the left thigh. The coagulation time was 187 minutes; retraction and character of the clot were normal. The bleeding time was two and one-half minutes. There was a moderate degree of hypochromic anemia; platelets were abundant. Capillary resistance was 10 cm. Hg. After obtaining a twenty-four-hour specimen of urine to determine the basal level of vitamin C excretion, daily administration of 200 c.c. of orange juice was begun. Although saturation with the vitamin

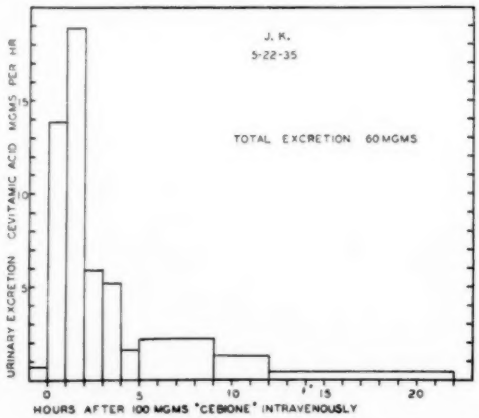

Fig. 1.—Hourly excretion of vitamin C after the intravenous administration of 100 mg. of cevitamic acid (Patient J. K.).

was accomplished (Table I), repeated hemorrhages into joints, muscle, and skin continued at intervals of two or three days. The capillary resistance remained unchanged. The intravenous administration of 100 mg. of cevitamic acid on May 22 resulted in a normal excretion curve (Fig. 1) and had no effect on the recurring joint and muscle hemorrhages. On May 28, after receiving 100 mg. of vitamin C daily for three weeks, a submucous hemorrhage into the larynx occurred, resulting in difficulty in breathing and swallowing, and in hoarseness. The coagulation time was 230 minutes; the capillary resistance had decreased to 6 cm. Hg. The intramuscular administration of 15 c.c. of normal human serum and the transfusion of 300 c.c. of citrated blood resulted in prompt cessation of the hemorrhagic manifestations for a period of about two weeks. Orange juice, which had been discontinued because of difficulty in swallowing, was resumed on June 15. During the next two months repeated hemorrhages occurred, requiring three transfusions. After each transfusion, bleeding temporarily ceased and the coagulation time diminished, on one occasion from 163 to 31 minutes, on another occasion from 180 to 15 minutes. It is apparent that in this patient saturation with orange juice and the administration of vitamin C intravenously had no effect on the bleeding, the coagulation time, or the capillary resistance.

CASE 2.—W. K., brother of J. K. At the age of six months a hemorrhage occurred in the cervical portion of the spinal cord, resulting in paralysis of all extremities for nine months. Neurologic residuals still persist in the form of ptosis of the left lid and scattered sensory

changes. The interval between hemorrhages of various parts of the body has never been more than a month. All extremities showed permanent deformities due to repeated joint hemorrhages. Two months before vitamin C studies were begun, there had been a massive hemorrhage into the muscles of the left thigh. As this subsided, occasional, small, subcutaneous hemorrhages occurred.

On May 23, 1935, the clotting time was 165 minutes; the clot retracted in normal fashion. The bleeding time was three minutes. There was no anemia; platelets were abundant. Capillary resistance was 17 cm. Hg. There had been no hemorrhagic manifestations for several days. Four hundred cubic centimeters of orange juice were added to the daily diet. Six days later, bleeding into the muscles of the right thigh began and continued for about ten days. The capillary resistance on three occasions during this period remained at 16 or 17 cm. Hg. The excretion of large amounts of vitamin C in the urine indicated that saturation with the vitamin had taken place at the time the bleeding began (Table I). The coagulation time on June 15, after almost three weeks of high vitamin C intake, was 130 minutes. Although orange juice was continued during the next two months, intermittent bleeding occurred from time to time. During this period two transfusions were required to control major hemorrhagic episodes.

CASE 3.—W. P., a thirty-seven-year-old man, had had repeated attacks of hematuria and hemorrhage into joints and muscles since early childhood. Both he and his brother were

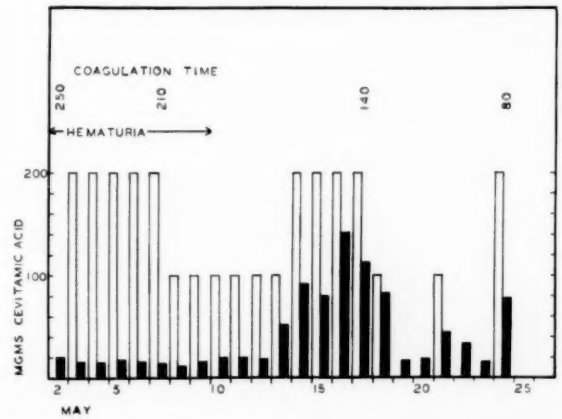

Fig. 2.—The daily intake and excretion of vitamin C in a patient with hemophilia (W. P.). The open columns represent cevitamic acid taken in the form of orange juice. The solid columns represent the twenty-four-hour excretion of cevitamic acid in the urine. The coagulation time is given in minutes.

known hemophiliacs. A maternal uncle had died of hemorrhage following an injury. April 30, 1935, the patient was readmitted to the hospital because of hematuria of ten days' duration. The urine contained gross blood and some albumin but showed no casts. There was no anemia. Blood coagulation time was 250 minutes; clot retraction was normal. Bleeding time was two and one-half minutes. Capillary resistance was 7 cm. Hg and remained between 7 and 10 cm. Hg during the period of observation. The intake and urinary excretion of vitamin C are shown in Fig. 2. No other therapy was given. The urine began to clear within a few days; there was no evidence of macroscopic or microscopic hematuria after May 9.

It is apparent from the chart that the hematuria cleared and the coagulation time decreased somewhat during the period of vitamin C administration. However, it cannot be stated with certainty that vitamin C was responsible for the improvement observed, inasmuch as previous episodes of hematuria had cleared within a comparable or shorter period of time without antiscorbutic therapy. It should be noted that the urinary excretion of cevitamic acid did not begin to increase until several days after the urine had completely cleared. Orange juice was continued after the patient was discharged from the hospital; on June 4 the coagulation time had increased from 80 minutes to 136 minutes. A few days later there was bleeding into the subcutaneous tissues of the scrotum.

The significance of the vitamin C excretion curve is not clear. Saturation apparently did not occur until after large amounts of orange juice had been given for ten days. In the majority of normal individuals, even after a diet partially deficient in vitamin C, increased excretion of cevitamic acid begins within two or three days after the addition of large amounts of orange juice to the diet. In this patient, after saturation had occurred, the response to alternate deprivation and addition of vitamin C was similar to that which has been observed in normal subjects. It is possible that factors as yet uninvestigated may have been responsible for the apparent delay in saturation. A similar, but more prolonged, delay in the excretion of cevitamic acid has been observed in a patient with hematuria due to acute hemorrhagic nephritis.

TABLE I

PATIENT	TWENTY-FOUR-HOUR EXCRETION OF CEVITAMIC ACID IN MILLIGRAMS			
	CONTROL	DURING ADMINISTRATION OF ORANGE JUICE		
	REGULAR DIET	SEVENTH DAY	FOURTEENTH DAY	TWENTY-FIRST DAY
J. K.	5.2	95.5	60.0	-
W. K.	11.7	121.0	136.0	128.5
W. P.	14.3	16.9	141.0	73.5
C. M.	31.2	57.4	-	72.5
F. M.	12.4	34.7	30.6	30.6
R. T.	19.0	49.5	105.0	-

CASE 4.—C. M., a twenty-eight-year-old man, was admitted to the hospital because of persistent bleeding following tooth extraction ten days before. A previous tooth extraction had been followed by persistent hemorrhage of ten days' duration. There had been no other significant hemorrhagic phenomena. His usual diet contained very little fresh fruit or leafy vegetables. The patient's brother, one maternal uncle, and three male maternal cousins were said to be "bleeders." The physical examination was essentially negative except for mild oozing of blood from the tooth socket. A moderate degree of hypochromic anemia was present. Platelets were abundant. Coagulation time was twenty-five minutes; clot retraction was normal. Bleeding time was six minutes. A diagnosis of mild hemophilia was made on the basis of the blood findings and the family history.

A compound pressure splint was applied to the bleeding area and 100 mg. of cevitamic acid were administered intravenously. Bleeding ceased within four hours.

Four days later 200 c.c. of orange juice were added to the daily diet. One week later, the bleeding time was six minutes, coagulation time twenty minutes. After three weeks of high vitamin C intake, the coagulation time was forty minutes. The anemia improved without other therapy, and the patient gained weight. He volunteered the information that since taking orange juice he had felt better than for many years. There was no change in the capillary resistance during the period of observation. The improvement in general health, the gain in weight, and the improvement in the anemia after adding orange juice to the diet, considered with the previous dietary history, suggest that this patient may have had mild vitamin C deficiency. Saturation with vitamin C, however, had no demonstrable effect on the coagulation time or the capillary resistance.

CASE 5.—R. T., a twenty-one-year-old man, had been known to have chronic thrombocytopenic purpura for at least three years. In addition to intermittent bleeding, there had been episodes of characteristic agranulocytic angina in 1932 and in 1933. In May, 1935, he had been free of hemorrhagic manifestations for about one year. During this period, however, there had been persistent thrombocytopenia, without other demonstrable morphologic blood abnormalities. On May 28, hematologic observations were as follows: hemoglobin, 16.2 gm. per 100 c.c.; red blood cells, 5,600,000 per c. mm.; white blood cells, 4,360 per c. mm., of which 58 per cent were neutrophilic granulocytes. Platelets were markedly diminished. Bleeding time was five and one-half minutes. Coagulation time was fifteen minutes; after twenty-four hours there was no retraction of the clot, which was very friable. Capillary resistance was 12 cm. Hg. Saturation with vitamin C was accomplished (Table I) by the addition of 400 c.c. of orange juice to the daily diet. After two weeks, the coagulation time was

nineteen minutes; the clot did not retract. Bleeding time was six minutes. No significant change had taken place in the hemoglobin, red blood cells, white blood cells or platelets. During the period of observation, the capillary resistance remained between 12 and 16 cm. Hg.

CASE 6.—F. M., a sixty-one-year-old carpenter, had been known to have chronic thrombocytopenic purpura for at least five years. His clinical course had been characterized by persistent granulocytopenia of varying degree and by episodes of bleeding and anemia. For at least a year prior to the vitamin C studies, there had been frequent episodes of bleeding from the nose and gums, interspersed with showers of petechiae and traumatic ecchymoses, so that at no time had he been free of hemorrhagic manifestations of one kind or another. On May 28, 1935, there were scattered, fresh petechiae in several areas and a recent ecchymosis on the right arm. Blood hemoglobin was 12.5 gm. per 100 c.c.; red blood cells numbered 4,430,000 per c. mm., white blood cells, 920 per c. mm. Differential count was as follows: eosinophiles, 1 per cent; stab neutrophiles, 1 per cent; segment neutrophiles 26 per cent; lymphocytes, 69 per cent; monocytes, 3 per cent. The platelets were markedly diminished. Bleeding time was eleven and one-half minutes. Coagulation time was nineteen minutes. There was no retraction of the clot in twenty-four hours; the clot was flabby and friable. Capillary resistance was 9 cm. Hg. The daily administration of 400 c.c. of orange juice resulted in the excretion of almost three times as much vitamin C as during the control period, indicating at least partial saturation with the vitamin. Frequent showers of petechiae and small ecchymoses continued to occur. If anything, hemorrhagic manifestations were more marked during the period of orange juice administration. After two weeks of forced vitamin C feeding, the blood findings were as follows: hemoglobin, 12.6 gm. per 100 c.c.; red blood cells, 5,010,000 per c. mm.; white blood cells, 1,160 per c. mm. Platelets remained markedly diminished. Bleeding time was twelve minutes. Coagulation time was twenty-three minutes, without change in the character of the clot. During the period of observation the capillary resistance remained at 9 or 10 cm. Hg. High vitamin C diet was continued for three months without change in the frequency or severity of the skin and mucous membrane bleeding.

DISCUSSION

Table I presents evidence that saturation with vitamin C was accomplished in each of the six individuals studied, with the possible exception of F. M. The apparent delay in saturation observed in W. P. indicates the need for further investigation of the factors which determine vitamin C excretion. Patients with hemophilia and with thrombocytopenic purpura apparently do not differ from normal individuals in their ability to excrete large amounts of ingested vitamin C. Confirmatory evidence of this is found in the report of Schroeder[7] who observed a normal response to the administration of 300 mg. of ascorbic acid in one patient with hemophilia. Finkle,[8] on the other hand, reports low values for vitamin C excretion in five cases of thrombocytopenic purpura and in four cases of metrorrhagia. It should be noted, however, that the previous dietary history of these patients is not mentioned and that results are expressed in terms of urinary concentration of vitamin C, rather than as the total amount excreted.

It is apparent from the case reports that saturation with vitamin C had no demonstrable effect on the hemorrhagic manifestations. In three of the patients with hemophilia and in one patient with thrombocytopenic purpura, hemorrhages recurred or continued during the period when large amounts of orange juice were being given. The other two patients were in symptomatic remission at the time the observations were made. No significant changes in the number of platelets, bleeding time, clotting time, character of the clot, or

in capillary resistance were observed. These results suggest that there is no characteristic abnormality of vitamin C metabolism and no constant therapeutic effect of antiscorbutic material in hemophilia or thrombocytopenic purpura.

It has been shown that the primary disturbance responsible for the hemorrhagic manifestations of scurvy is a separation of the cement substances binding the vascular endothelial cells.[9] Wolbach and others[10, 11] have demonstrated that the immediate effect of the administration of orange juice or ascorbic acid in experimental scurvy is the prompt deposition of intercellular material. Preservation of the integrity of the intercellular cement substance is apparently a specific function of the antiscorbutic vitamin. It is noteworthy that disturbances of the intercellular cement substance have not been described in hemorrhagic lesions other than those due to scurvy. Theoretically, therefore, vitamin C might be expected to have no effect on the capillary bleeding of the other hemorrhagic diatheses unless an associated vitamin deficiency of some degree were present.

Instances of capillary hemorrhage due to mild or "subclinical" scurvy may be more frequent than has hitherto been suspected. We have recently observed three individuals with gingival bleeding thought to be due to relative vitamin C deficiency. Consumption of vitamin C containing foods had been curtailed because of special diets or individual preferences. In each instance there was a history of bleeding from the gums, occurring both spontaneously and after brushing. The gums appeared dusky, mottled, soft, and edematous. There were no symptoms or signs of hemorrhage from other sites. Bleeding time, coagulation time, blood counts, and platelets were normal. Large amounts of vitamin C were supplied in the form of 400 c.c. of orange juice daily. Although there was no significant change in the capillary resistance, the gingival bleeding diminished almost immediately and ceased entirely within a few days. Cessation of the bleeding was accompanied by an increase in the urinary excretion of cevitamic acid. In two of these individuals the bleeding returned when orange juice was omitted from the diet, disappeared when large amounts of vitamin C were again given. In another instance the subcutaneous hemorrhages, which had regularly followed desensitizing doses of pollen extract given to a young woman with hay fever, permanently disappeared when adequate amounts of orange juice were added to the diet.

There are undoubtedly other types of capillary bleeding which may be associated with inadequate vitamin C intake. Hemorrhages in infantilism and marasmus have been observed as a consequence of vitamin deprivation.[9] The "symptomatic" or "simple" purpuras, in which morphologic blood changes are not demonstrable, should be studied from this point of view. Many patients with gastrointestinal and other diseases are given diets which contain inadequate amounts of fresh fruits and leafy vegetables. Such individuals might easily develop a relative vitamin C deficiency unless care is taken to provide orange juice or other suitable antiscorbutic material. The possible relationship of the bleeding of peptic ulcer to vitamin deficiency has not been investigated. Harris and Ray[4] have suggested a test for subclinical scurvy which

depends on the urinary excretion of cevitamic acid after a massive test dose of vitamin C. The results of the application of this test to various types of capillary bleeding will be of interest.

SUMMARY

1. The effect of large doses of orange juice has been studied in four patients with hemophilia and in two patients with chronic thrombocytopenic purpura.

2. Saturation with vitamin C had no effect on the hemorrhagic manifestations, the blood picture, or the capillary resistance.

3. It is suggested that favorable therapeutic effects may be expected from the administration of vitamin C only in those instances of capillary bleeding which are associated with some degree of vitamin C deficiency.

NOTE: Since the preparation of this communication, Wright and Lilienfeld[12] have reported observations on the effect of the administration of cevitamic acid to three patients with purpura and two patients with hemophilia. They expressed the opinion that its value in the treatment of thrombocytopenic purpura or familial hemophilia is doubtful. Miller and Rhoads[13] observed a persistent rise in the number of thrombocytes and complete relief of symptoms following the administration of cevitamic acid to four patients with idiopathic thrombocytopenic purpura. In two instances, clinical improvement and increase in the number of thrombocytes were associated with an increased urinary output of ascorbic acid.

The authors wish to express their appreciation to Dr. Samuel W. Clausen for his interest in this study.

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A STUDY OF DECAMETHYLENEDIGUANIDINE BITARTRATE (ANTICOMAN)*

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IN 1931 and 1932, there appeared in the German literature[1] descriptions of a proprietary substance called "anticoman" recommended to be administered orally in the treatment of diabetes mellitus, to reduce hyperglycemia. Prior to this time, attempts[2] had been made to produce a substance which, if administered orally, would have the same effect as insulin. Many of these products have been shown to be rather toxic or ineffective. A well-known example of these attempts is "synthalin," which is a guanidine preparation.

In 1933, the chemical nature of "anticoman" was revealed to be decamethylenediguanidine bitartrate. It is soluble in water and gastric juice, and may be prepared by first making a basic compound by melting a polymethylenebisguanidine with the rhodonate of guanidine at 130° to 160° C. and treating with alkali hydrate or carbonate. These basic compounds are then caused to react with tartaric acid.[3]

TOXICITY FOR MICE

Method.—We first used a water solution of decamethylenebisguanidine bitartrate, the concentration of which was 5 mg. of the salt in 1 c.c. of the solution. In the experiments, we used white mice weighing between 15 and 20 gm. All injections were made subcutaneously. The mice were weighed daily, and the injections adjusted accordingly. Results are found in Table I.

TABLE I

MOUSE	DOSE	DOSE IN MILLIGRAMS PER 100 GM. OF MOUSE	RESULTS
1	1 c.c.	34 mg.	Dead in 2 hours
2	0.75	23 mg.	Dead in 30 minutes
3	0.50	15 mg.	Dead in 1 hour 15 minutes
4	0.25	7 mg.	Dead in 2 hours

Using a solution which contained 1.25 mg. of the salt in 1 c.c., we obtained the results as shown in Table II.

Discussion.—When large doses are given, as in Mice 1 to 4 inclusive, about one hour previous to death, a convulsive type of reaction could be elicited by the slightest stimulus. This may be similar to the symptom complex observed in cats, produced by dimethyleneguanidine, and almost identical with spas-

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mophilia in children. This might be explained on the basis that guanidine produces symptoms similar to parathyroid tetany.[4] From the results obtained with mice, it is to be noted that 2.25 mg. of decamethylenediguanidine bitartrate per 100 gm. of mouse can be injected and the animal will remain alive for at least twenty-four hours. However, a dose of about 1.0 mg. of salt per 100 gm. of mouse is the largest dose that can safely be injected daily for any appreciable period of time.

TABLE II

MOUSE	DOSE	AVERAGE DOSE IN MG. PER 100 GM. OF MOUSE		RESULTS
8	1.0 c.c.	6.5 mg.		Dead in 3 hours
7	0.75	5.7		Dead in 20 hours
6	0.5	3.1		Dead in 20 hours
12	0.3	2.24		Dead in 48 hours
5	0.25	1.8		Living
11	0.3	2.5	Injected daily for two days	Died
10	0.3	2.2	Injected daily for three days	Died
9	0.3	2.0	Injected daily for three days	Died
17	0.3	2.2	Injected daily for two days	Died
16	0.3	2.0	Injected daily for three days	Died
21	0.3	2.0	Injected daily for three days	Died
13	0.3	1.8	Injected daily for five days	Died
14	0.15	1.3	Injected daily for four days	Living
15	0.3	2.2	Injected on alternate days	Living after 3 injections
18	0.3	1.9	Injected on alternate days	Died after 3 injections
19	0.15	0.95	Injected daily for 17 days	Died
20	0.15	1.04	Injected daily for 17 days	Died

Conclusions from these experiments on mice: The largest dose of decamethylenediguanidine bitartrate that can be used for subcutaneous injections is 2.25 mg. per 100 gm. of mouse for a single injection; 1.0 mg. per 100 gm. of mouse for repeated daily injection.

TOXIC AND GLYCEMIC EFFECTS ON NORMAL RABBITS

Methods.—The rabbits used in these experiments were starved for a period of twenty-four hours previous to the administration of the drug, and no food was given during the experiment. The drug was administered through a stomach tube. One cubic centimeter of blood was drawn from the marginal ear vein for sugar determination, a modification of the Folin-Wu method being used.[5] Results are found in Table III.

The effect of the salt on the blood sugar of rabbits when injected subcutaneously was then observed. A 4 per cent solution was used. Results are found in Table IV.

Observations.—Rabbits 17 and 29 died in convulsions when their blood sugar dropped to 36 and 22 mg., respectively. Microscopic sections were made of the kidneys and livers of Rabbits 15, 17, and 29. Tissues were taken immediately after death.

TABLE III

RABBIT	WT. IN KG.	DOSE PER KG.	NORMAL	BLOOD SUGAR IN MG. PER 100 C.C.								
				1 HR.	2	3	4	5	7	24	28	54
1	2.2	Control	105	81	91	80				140		
2	2.0	20 mg.	118	85	94	72				85		
3	2.4	20	111	103	112	96				69		
4	2.3	Control	119					99	117			
5	1.9	20	113					93	103			
6	2.5	20	133					129	129			
7	2.6	Control	129	110	102		120	116		94	91	
8	3.0	24	116	120	118		105	109		98	89	
9	2.3	24	120	112	116		115	107		95	96	
10	2.6	24	127	108	120		110	113		94	84	
11	2.5	30	110	98	113		104	113		86	82	
12	2.3	30	119	102	111		114	93		75	76	
13	1.6	40	104	78			83				83	
14	2.8	40	98						86	99		
15	1.4	50	100	58			57		Died			
16	2.0	50	101						80.3		Died	

TABLE IV

RABBIT	WT. IN KG.	DOSE PER KG.	NORMAL	BLOOD SUGAR IN MG. PER 100 C.C.													
				1 HR.	2	6	8	15	17	21	22	23	24	26	28	32	42	48
17	3.0	6 mg.	123	147							50		36					
18	3.1	9	109	136														
19	2.8	Control	89	94							109							
20	2.4	3	104		121	130	145							108	96	88		
21	3.1	5	106		119	102	132							112	121	111		
22	2.8	Control	105		95	98	121							104	97	100		
23	2.1	Control	105					90	88	97		104					94	100
24	2.3	3	103					118	129	125		125					125	131
25	2.8	5	98					114	94	108		101					116	121
26	2.3	5	110	146	106	119							115	128		127		
27	3.2	6	108	121	77	113							106	104		119		
28	2.4	7	104	141	101	92							73	77		74		
29	2.0	9	103	51	22													

Microscopic examination revealed a marked necrosis and desquamation of the tubular epithelium of the kidney; a condition similar to that observed in mercuric poisoning. This was especially noted in the tissue from Rabbit 15. Sections from the livers did not show any pathology. Tissues were stained by hematoxylin and eosin.

SUMMARY

The results as shown in Table III indicate that there is little effect on the blood sugar when small doses of the salt are administered orally. However, with a dose of 50 mg. of anticoman per kilogram of rabbit, the blood sugar dropped markedly, and the rabbits died. The next smaller dose of 40 mg had only a very slight hypoglycemic effect. This seems to indicate that the glycemic effect

is obtained only when the toxic dose is reached. In Table IV, the results show that doses up to and including 5 mg. of decamethylenediguanidine bitartrate per kilogram of rabbit subcutaneously have practically no hypoglycemic effect on normal rabbits when observed over a period of forty-eight hours. When the dose is raised to 6 mg. of salt per kilogram of rabbit, the hypoglycemic effect may be so great as to produce convulsions and death; or, there may be only a slight hypoglycemia, apparent after six hours, but this disappears by the eighth hour. Above 6 mg., marked hypoglycemia is produced and the animal dies in convulsions.

CONCLUSIONS FROM EXPERIMENTS ON RABBITS

1. On oral administration, 50 mg. of decamethylenediguanidine bitartrate per kilogram of normal are lethal. Forty milligrams produce a slight hypoglycemia, smaller doses have practically no effect.

2. On subcutaneous injections, doses over 6 mg. per kilogram are effective in producing hypoglycemia but are fatal. Six milligrams in some cases may or may not produce hypoglycemia, but below this dose, the salt is ineffective.

3. Histologic examination shows that the effect of the drug is to produce a tubular nephritis, similar to that obtained in mercuric poisoning.

4. From these results, we cannot expect the drug to be of value in clinical cases.

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LABORATORY METHODS

THE PRACTICAL VALUE OF EMPLOYING MORE THAN ONE LABORATORY PROCEDURE IN THE SERODIAGNOSIS OF SYPHILIS*

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FOR some time we have been employing Kolmer's 2-tube complement fixation method as a routine procedure, together with Kahn's standard test upon request, in the serodiagnosis of syphilis. To date we have accumulated comparative reactions on more than 50,000 specimens.

It is our purpose to set forth in this paper these comparisons with the view of presenting the practical value of employing more than one kind of procedure. No attempt has been made to obtain the diagnoses of the clinicians with the exception of the first 10,000 specimens under consideration.

It is well, we think, to describe briefly the procedure employed. Although appreciating the superiority of Kolmer's more recent 3-tube method, the volume of our work, together with limitation of personnel and equipment, has necessitated the continuation of his former method. Serum inactivated for thirty minutes is employed in doses of 0.1 c.c. Natural antisheep hemolysin is not removed. Titrations of both hemolysin and complement (1-30) are made daily. Ten units of antigen are used. Primary incubation is conducted at 6° to 8° C. for fourteen to eighteen hours followed by water-bath for ten to fifteen minutes. Secondary incubation is in water-bath for one hour. Reactions are read immediately.

The Kahn 3-tube standard test is performed on the same day the complement fixation test is set up. Standardized antigen is obtained from the Michigan Department of Health.

Table I

Classification of Wassermann and Kahn Reactions on 50,000 Specimens

		WASSERMANN					
		4+	3+	2+	1+	±	Neg.
KAHN	4+	6157	203	114	165	77	277
	3+	861	88	120	101	51	305
	2+	208	51	82	122	38	375
	1+	96	18	42	86	43	306
	±	65	7	10	24	38	203
	Neg.	215	39	66	105	92	40,532

*From the Department of Serology, Georgia State Department of Health.
Received for publication, February 7, 1936.

In cases of disagreement both tests were repeated in order to rule out technical error. Table I gives the results of these comparative examinations in tabulated form. It will be noted that 277 specimens showed four-plus Kahn and negative Wassermann, whereas 215 specimens gave four-plus Wassermann and negative Kahn reactions.

It must be borne in mind that the higher the percentage of positive reactions encountered in routine work, the higher the discrepancy in reactions is likely to be. We would not expect to find as many disagreements of results in nonsyphilitic as in syphilitic patients. The average percentage of positive reactions in routine examinations over the period of time covered in this study ranged from 18 to 20 per cent. Employing the same procedures, laboratories would probably find fewer or more discrepancies dependent upon the average percentage of positive reactions.

We find that almost 1 per cent of the 50,000 specimens showed four-plus by one and negative by the other test, with the Kahn showing slightly more positives. These represent about 8 per cent of the four-plus reactions in which both tests were in agreement.

TABLE II

SUMMARY OF DISAGREEMENTS BETWEEN THE WASSERMANN AND KAHN TESTS

Positive Kahn—negative Wassermann	957
Positive Wassermann—negative Kahn	320
Positive Kahn—doubtful Wassermann	554
Positive Wassermann—doubtful Kahn	238
Doubtful Kahn—negative Wassermann	509
Doubtful Wassermann—negative Kahn	197

The disagreements are summarized in Table II by the usually accepted scheme of representing four-, three-, and two-plus reactions as positive, and one-plus and plus minus as doubtful. The Kahn test gives the greater number of positive reactions.

Mention was made above that we sought a study of the clinical data in the disagreements encountered in the first 10,000 specimens. Questionnaire cards relating to the history and symptoms were attached to the reports. The physicians cooperated well indeed, returning 93.4 per cent of the cards. Clinical diagnoses usually pointed to the positive results in these cases of disagreement between the two tests.

TABLE III

ANTICOMPLEMENTARY REACTIONS

KAHN	4+	3+	2+	1+	±	−	TOTAL
	302	69	38	8	10	122	549

ANTICOMPLEMENTARY REACTIONS

The Kahn test was made on all anticomplementary Wassermann reactions encountered in routine work during this study. Table III gives the results of these reactions. It will be noted that the vast majority of the anticomplementary reactions gave positive Kahn results. This causes one to wonder as to the rela-

tionship of such to specific reactions. In our experience the majority of anticomplementary reactions obtained with two units of complement yield apparently positive reactions when repeated with three units of complement and are positive by the Kahn test.

TABLE IV

SHOWING CONSISTENCY OF RESULTS OBTAINED WITH WASSERMANN AND KAHN TESTS IN DIFFERENT GROUPS OF SPECIMENS

	FIRST 10,000	NEXT 20,000	NEXT 20,000	TOTAL 50,000
1. *Identical Agreement* No variation in terms of plus	91.1	91.4	91.6	91.4
2. *Complete Agreement* 4+, 3+, and 2+ = positive. 1+ and ± = doubtful. Positive, doubtful or negative by both tests	94.2	94.4	94.9	94.6
3. *Relative Agreement* Positive or negative by one test and doubtful by the other	3.4	2.8	2.8	2.9
4. *Disagreements* Positive by one test and negative by the other	2.4	2.8	2.3	2.5

Table IV is presented to demonstrate the consistency of the agreement between the two tests employed. In the first column are given the percentages for the first 10,000 specimens. The second column represents the following 20,000 and the third column the last 20,000 specimens. The fourth column gives the average for the entire group.

It will be noted that there is less than 1 per cent variation in each of the groups presented. We feel that this consistency is significant of the result of controlling one test by means of another. There have been instances when we found the results by the two tests diverging more than usually. Investigation revealed the trouble somewhere along the line, which, when remedied, resulted in a return to the usually expected agreement. These difficulties have been experienced in the performance of both the complement fixation and Kahn tests.

We believe that herein lies the practical value to the laboratory in employing more than one procedure. There may be little to choose as to efficiency between a good complement fixation and a good flocculation method, provided they are carefully controlled. The question is, what is the best method to control them? The controls set up with each test are highly desirable, but they may not reveal errors of technicians or gradual changes in reagents. For instance, if an antigen should lose antigenic properties or become anticomplementary, it is reasonable to suppose these changes develop gradually and may exert influence over the results of the test before they are detected by the controls. Then again the specificity of a new lot of antigen may vary from that of the lot in use, even though the titer with a particular pooled serum may be as good or even superior.

The employment of two tests routinely, as recommended by the Health Organization of the League of Nations, and others, is to be desired. However, some laboratories find it impractical to execute these recommendations because of economic reasons. The preference of tests to use routinely and for check pur-

poses will usually rest with the serologist as determined by his experience. It occurs to us that this purpose can best be served by choosing a representative test from both the fields of complement fixation and flocculation.

Our conclusion is that aside from the added information the laboratory may be able to give the physician, the employment of an additional procedure, at least to such an extent that it will serve as a check and control on the other, is conducive to better work in the routine serodiagnosis of syphilis. This conclusion, of course, is based upon the assumption that conditions will permit the employment of a supplementary test without in any way detracting from the usual care and accuracy demanded in serologic work.

A PRACTICAL METHOD OF MEASURING AND RECORDING SKIN TEST REACTIONS*

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THE interpretation of skin tests is generally based on size in centimeters and degree of inflammatory reaction. The methods of determining the size of skin reactions have been most diverse, varying from the employment of rulers and calipers to mere guesswork. The method of recording has been usually word description or the employment of positive and negative signs. It has been our purpose to devise a simplified uniform method of measurement and recording with the view of increasing the rapidity, accuracy, and efficiency of such interpretation. While we have employed the following technic only in regard to Schick and Dick reactions, it could easily be adapted to any form of skin testing.

Procedure.—A thin glass 4 cm. square is used upon which there are three circles. The small inner circle is 1 cm. in diameter, the second circle 2 cm. in diameter, and the outer and largest circle is 3 cm. in diameter. The glass is placed over the site of reaction so that the small circle is in the middle of the reacting area. By looking through the glass at the skin, the extent of the reaction is immediately given in terms of centimeters. The appearance of the glass is seen in Fig. 1.

In recording the reaction, a rubber stamp (see Fig. 1) is used which contains three circles of the same size as described above. Each time a reading is made, by use of the stamp, a diagram is imprinted on the card of the patient and the date of reading recorded below the diagram. The reaction is recorded schematically on the diagram by the employment of an arbitrary standard (see Fig. 2).

Using the standard glass, therefore, and the standard of color interpretation, an accurate schematic representation may be immediately transferred to the patient's record which can readily be visualized at any time, and can more easily be visualized than a word description of the same reaction. One who had

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not seen the patient could easily determine the type of reaction by a very rapid scanning of the patient's record inasmuch as there would immediately meet his eye a daily schematic representation of the reaction which could be correlated at a glance.

For illustration, and to exemplify the method of recording, duplications of actual Schick reactions are given in Fig. 3 and Dick reactions in Fig. 4. An associated word interpretation will be given in a few cases, which, of course, is not necessary when one is familiar with the method.

The record of Case 1 in Fig. 3 would be interpreted as follows: At the end of twenty-four hours there was a dark pink area 0.5 cm. in diameter surrounded by a light pink area 2 cm. in diameter. At the end of forty-eight hours, the dark pink area had increased to 1 cm. and the light pink area to 3 cm.

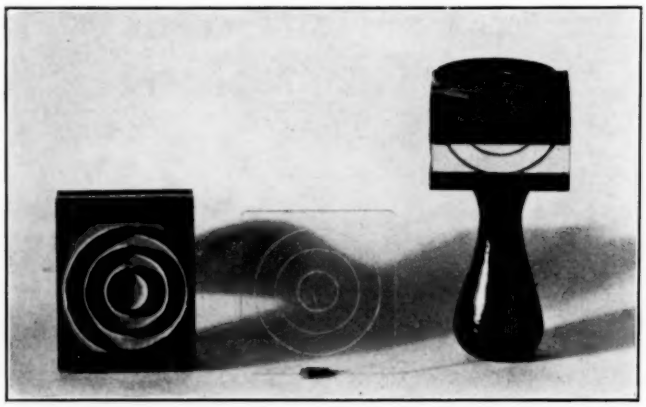

Fig. 1.—The measuring glass and recording stamp.

Fig. 2.—Schematic standard employed in recording color reactions.

At the end of seventy-two hours the 1 cm. dark pink area became a deep red and the light pink area remained approximately the same. At the end of ninety-six hours, the light pink area had faded but the deep red area had increased to 2 cm. At the end of the seventh day there was a 2 cm. brownish red pigmented area.

The record of Case 4 in Fig. 3 would be interpreted as follows: At the end of twenty-four hours there was a dark pink area 0.5 cm. in diameter surrounded by a light pink area 3 cm. in diameter. At the end of forty-eight hours the dark pink area had become red and increased to 1 cm. in diameter. The light pink area had become dark pink and increased to 3.5 cm. At the end of seventy-two hours there remained only a light pink area 3.5 cm. in diameter. At the end of ninety-six hours there was complete fading.

In Fig. 4 the records would be interpreted as follows: Case 1, a light pink area 2 cm. in diameter; Case 2, a dark pink area 1.5 cm. in diameter; Case 3, a dark pink area 3 cm. in diameter; Case 4, a dark pink area 2 cm. in diameter.

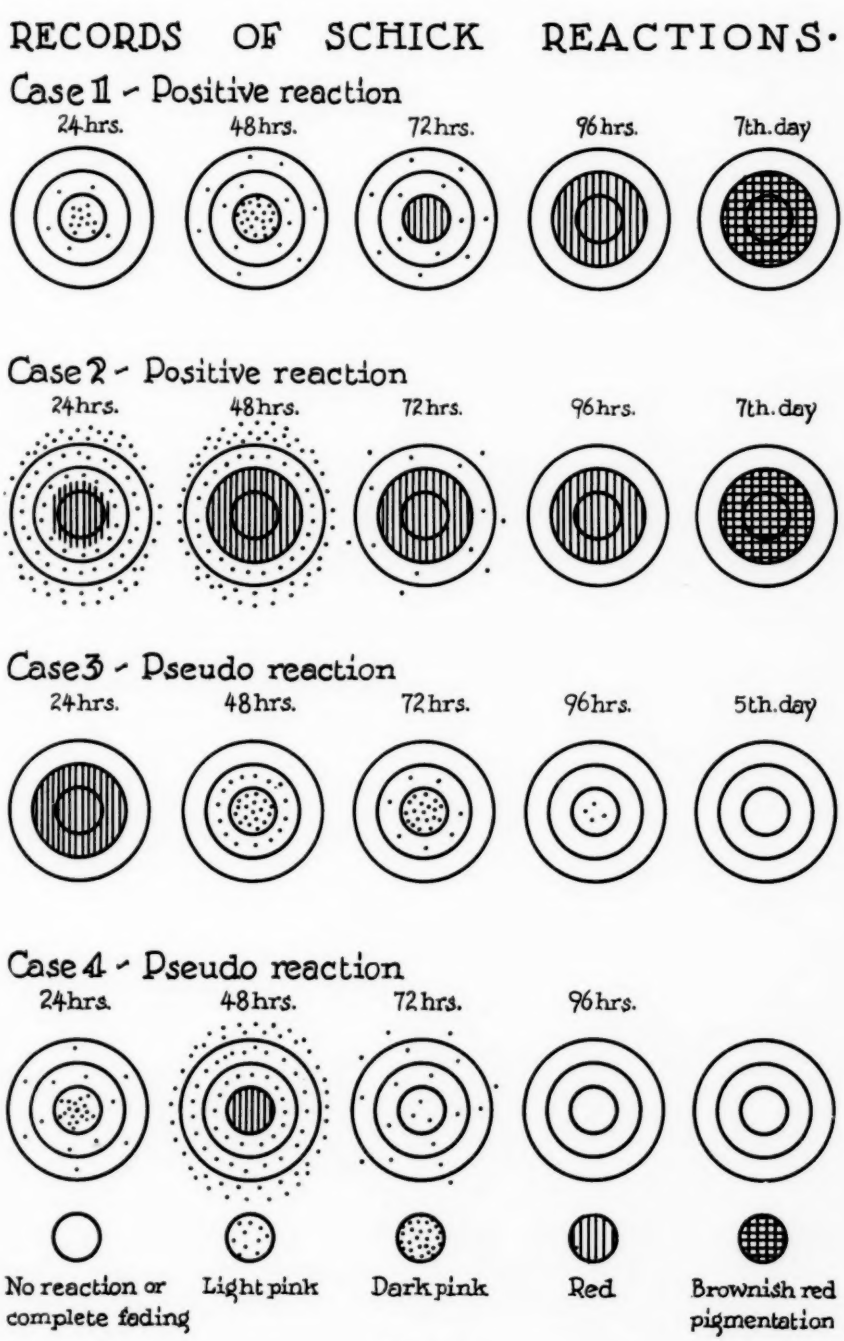
RECORDS OF SCHICK REACTIONS.
Case 1 - Positive reaction
24 hrs.
48 hrs.
72 hrs.
96 hrs.
7th. day
Case 2 - Positive reaction
24 hrs.
48 hrs.
72 hrs.
96 hrs.
7th. day
Case 3 - Pseudo reaction
24 hrs.
48 hrs.
72 hrs.
96 hrs.
5th. day
Case 4 - Pseudo reaction
24 hrs.
48 hrs.
72 hrs.
96 hrs.
No reaction or complete fading
Light pink
Dark pink
Red
Brownish red pigmentation

Fig. 3.

DISCUSSION

The above-described method of measuring and recording skin tests (Schick and Dick reactions) has been used by us for three years on medical students with success. In 1934 this method was employed by the Division of Communicable Diseases of the Illinois Department of Public Health in Schick testing 140 children with excellent results. In 1933 in order to see whether the apparatus employed by two individuals would produce uniform results in the same

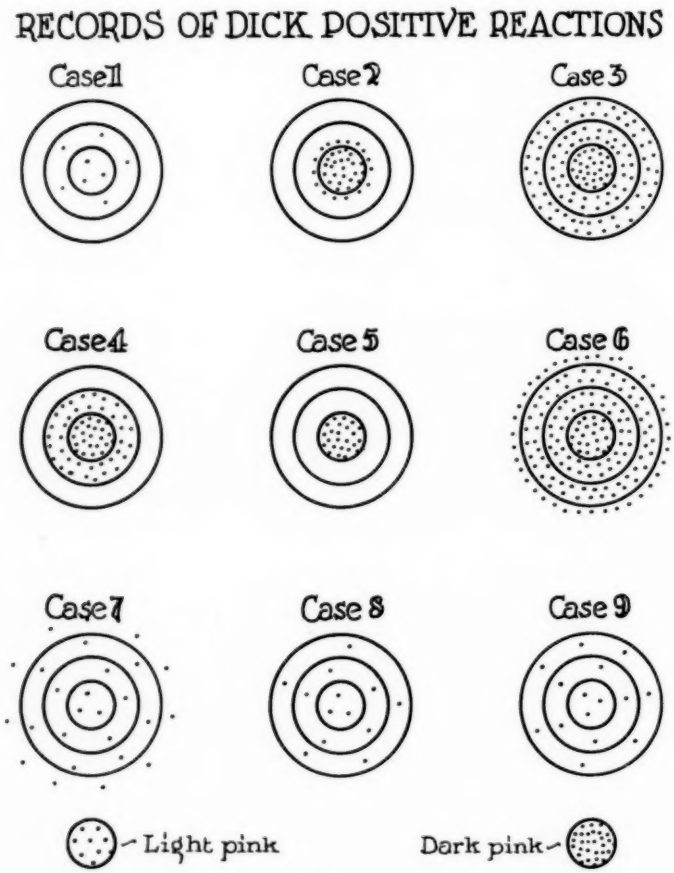

Fig. 4.

skin test interpretations, in a series of Schick and Dick tests, independent readings and recordings were made by two of us and when the final interpretations had been completed, a comparison of the two sets of records was made. Identical results were obtained in regard to the size of the reacting area. In regard to recording, the records tallied accurately, excepting in one or two instances where there was a difference of opinion as to whether the reacting area was light pink or dark pink. This did not alter the diagnosis of positive or negative but made a difference in the degree of positivity.

SUMMARY

1. A simplified and practical method of measuring and recording skin tests has been presented.

2. It has been used on adults and children with excellent results.

3. In the hands of different individuals, it produces strikingly similar results.

4. While this method has only been employed in Schick and Dick testing, it could be readily adapted to any form of skin testing.

CELL GROUP IDENTIFICATION OF DRIED BLOOD SPOTS AND TRACES OF SALIVA*

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THE principles of inheritance of "blood" group-specific factors are well worked out, and their forensic application in affiliation cases is receiving growing recognition.[1, 2, 11, 12]

The subject of group identification of dried blood spots has received little recognition in this country. Landsteiner in his first paper suggested the use of blood groups in the identification of human blood for medicolegal purposes, and with Richter, in 1903, experimentally demonstrated the feasibility of this suggestion.[3] This original work was based on the assumption that isoagglutinins are still present in dried blood and that autoagglutination is not a natural occurrence; hence, if an extract of a bloodstain is found to agglutinate the corpuscles of a given individual the stain cannot be due to this person's blood.[4] Since that time considerable work on this subject has been done in European countries. Through the initiative of Lattes[4] this work gained judicial recognition in Italy in 1916. Other case reports have come from Russia, France, and Germany.[4]

Müller[5] extracted bloodstains with dilute saline solution and by vacuum distillation obtained a concentrated extract which was then tested with known cells for its agglutinin content. He claimed 70 per cent results with stains up to eighteen months old. Levine[6] reports the demonstration of agglutinins in a Group 0 stain four years old. Holzer[7] found that after the first week attempts to demonstrate agglutinins gave clear-cut results in only 50 per cent of the cases. Schiff and Higuchi used a test for agglutinogen content based upon the absorption of agglutinins from a standard serum when exposed to dried blood. Holzer describes a quantitative modification of this test; he titrates the serum exposed to the bloodstain, and a portion of the serum exposed to a control portion of unstained material, using a capillary pipette to prepare progressively doubled dilutions on a special glass plate. Lattes[8] finds the quantities used by Holzer

*From the Central Laboratories, Indiana University Medical Center.
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suitable for measurement in graduated pipettes and titration in tubes; he recommends the use of absorbing serum in a dilution equal to one-sixteenth of its end-titer.

Jadin[9] has recently published results obtained by using the complement fixation test of Hirszfeld and Amzel.

Lattes[8] has pointed out that the conception of blood groups is rapidly becoming obsolete in the face of the newer conception of "cell groups" since group-specific factors (agglutinogens) are found in practically all cells (normal or pathologic), fluids and secretions of the body.

For further discussion of group-specific factors in body organs see Landsteiner and Levine[10] and Wiener.[11]

During the past year I have used various procedures suggested by European workers. By combining the extraction described by Jadin and the reconcentration suggested by Müller, and by testing for agglutinins and agglutinogens (agglutinin absorption and titration after the manner of Holzer), excellent results were obtained when adequate amounts of material were used. With marginal amounts the results were unsatisfactory. For the examination of small amounts of material a more sensitive technic was needed.

The following technic of agglutinin fixation was devised and has been found useful. The principle is the same previously employed but the amounts and methods afford higher sensitivity combined with greater speed and facility.

TECHNIC

Demonstration of Agglutinogens.—Principle: This demonstration depends upon specific fixation of agglutinins, contained in a standard serum, as a result of exposure to agglutinogens in the material being examined. This fixation is made apparent by finding upon subsequent titration that the titer of the test serum is reduced.

Choice of a Standard Serum: A Group 0 serum which has a strong titer approximately equal for A and B cells is required. Preliminary tests with 1:10 dilution upon slides will give a fair idea of suitability. If these tests seem satisfactory, the serum should then be titrated as follows:

a. Prepare two series of progressively doubled dilutions (0.25 c.c. in 8 by 1.4 cm. test tubes).

b. To each tube of the first series add 1 drop of an approximately 2 per cent suspension of A cells.

c. To each tube of the second series add a similar portion of B cells.

d. Shake to mix.

e. Incubate five minutes at 20 to 25° C.; centrifuge three minutes at high speed.

f. Shake to loosen the cells from the bottoms of the tubes and examine with a concave microscope mirror for agglutination.

g. Record the findings and the end-titer for A and B cells.

Agglutinin Fixation: 0.2 sq. cm. of the stained cloth (conveniently measured as ⅛ of a disc 14 mm. in diameter) is placed in a test tube (8 by 1.4 cm.) and thoroughly wet by one drop of the standard Group 0 serum. The tube is incubated forty minutes at 37° C.

At the end of that time sufficient saline solution (0.85 per cent) is added to secure a dilution of the serum equivalent to one-sixteenth of its end-titer. For example, if the end-titer of the serum for A and B cells is 128, then a dilution of 1:8 is secured by the addition of seven drops of saline. The cloth is washed, pressed out, and removed from the tube by use of a glass rod.

Three drops of the resulting fluid are placed in each of two test tubes. To one of these (labeled AT) is added one drop of a 2 per cent (approximately) suspension of A cells; to the other (BT) a similar portion of B cells is added.

Simultaneously an equal amount of the cloth which is not stained is treated in exactly the same way. The tubes (AC and BC) containing the diluted serum which has been exposed to the unstained cloth serve as controls for the test proper.

The four tubes are incubated at 20 to 25° C. for five minutes, then centrifuged at high speed for three minutes. They are removed from the centrifuge with care not to agitate them until they may be closely observed by means of a concave microscope mirror. AT and AC are shaken gently and the amount of agglutination in each is carefully compared; then BT and BC are examined.

Comparative Titration: If agglutination is present in both tubes, i.e., test and control, the maneuver of comparative titration is employed. This consists of holding the two tubes in one hand and, with wrist motion only, shaking them, gently at first then harder if necessary, to break up the clumps. The tubes should be shaken freely in the air so as not to strike against anything. The resistance of the clumps to shaking is proportional to the amount of agglutination and affords a comparative index of the titer of the fluid in the two tubes. If the clumps in the two tubes show an equal resistance to shaking, the titer of the test fluid has remained unchanged. If the clumps in the "T" tube disappear while the control still shows 2+ agglutination, it indicates a marked reduction in the titer of the test serum and the presence of the corresponding agglutinogen in the stained cloth. Not infrequently when there is no reduction in titer in the "T" tube, it shows more agglutination than its control; this is probably due to the agglutinin content of the bloodstain which in this circumstance tends to increase rather than to decrease the titer.

In the absence of agglutination the cells usually float free with the slightest agitation. Occasionally they adhere to the bottom of the tube and require considerable shaking to loosen; whenever they loosen they float free without clumps. This difficulty is usually the result of a light cell suspension.

Recentrifugalization and rereading are frequently helpful if the first reading is not clear cut. Microscopic readings are essentially noninforming after one has gained some experience with the concave mirror.

Demonstration of Agglutinins.—The technic used here for this purpose differs in no essential way from that used by Lattes and others.

A portion of the stained material is extracted with the smallest possible amount of 0.25 per cent saline solution. A drop of the extract is placed on each of three cover slips and allowed to evaporate to dryness. Second and third drops may be superimposed.

To one of these is added a drop of a suspension of A cells (approximately 2 per cent) sufficiently small that it is contained within the rim of the dried extract. The cover slip is inverted and placed upon a hanging drop slide. B cells are added to the second cover slip and 0 cells to the third.

The slides are incubated at 20 to 25° C. Final readings should not be taken before eight or twelve hours.

Agglutination of 1+ or more with both A and B cells indicates Group 0; agglutination with only the B cells indicates Group A; agglutination only with the A cells indicates Group B. The failure to observe agglutination is indicative of Group AB; but this is of little significance unless the stain is known to be less than ten days old. The 0 cells serve as a control against pseudo- and hetero-agglutination.

EXPERIMENTAL RESULTS

To determine the efficacy of these methods under unknown conditions, blood and saliva stains were prepared in the hospital admitting room and sent to the laboratory labeled only by serial number. The bloodstains upon freshly laundered white shirt cloth were sealed in envelopes by the various individuals

supplying the blood, thus securing the saliva specimens. Grouping tests upon fresh cell suspensions were made by another worker who withheld results until completion of the tests on the stains. The results of these examinations are recorded in Table I.

TABLE I

DIAGNOSES OF UNKNOWN SPECIMENS FROM THIRTY INDIVIDUALS

Specimens 2-3 weeks old when tested. Figures based on single examination. Saliva specimens taken from gummed flaps of the envelopes.

SPEC. NO.	BLOODSTAINS		FINAL DIAGNOSIS	SALIVA STAINS	KNOWN GROUP
	AGGLUTININ CONTENT	AGGLUTININ FIXATION		AGGLUTININ FIXATION	
1	?	O	O	O	O
2	O?	O	O	O	O
3	O?	O	O	O	O
4	AB?	O	O	O	O
5	A	A	A	A	A
6	O?	O?	O	O	O
7	A	O*	A	O*	A
8	O?	O	O	O	O
9	O	O	O	O	O
10	O	O	O	O	O
11	A	B*	?	A	A
12	A	A?	A	A	A
13	?	O	O	O	O
14	B	B	B	O*	B
15	O	O	O	O	O
16	A	O*	A	A	A
17	O?	O	O	O	O
18	A	O*	A	A	A
19	?	A?	A?	A	A
20	AB, O?	AB, O?	AB?	O*	AB
21	O	O	O	O	O
22	O	O	O	O	O
23	?	A, O?	?	A, O?	O
24	O	O, B?	?	O	O
25	A, O?	O	?	O	O
26	O?	O	O	O	O
27	O	O	O	O	O
28	O	O	O	O	O
29	O?	O	O	O	O
30	O	O?	O	O	O
Correct	16(53.3%)	19(63.3%)	24(80.0%)	26(86.7%)	
Inconclusive	14(46.7%)	7(23.4%)	6(20.0%)	1(3.3%)	
*Misdiagnosed	0(0.0%)	4(13.3%)	0(0.0%)	3(10.0%)	

On the basis of a single examination of the thirty bloodstains, 16 (53.3 per cent) were correctly diagnosed by testing for agglutinin content. In 14 (46.7 per cent) the results were inconclusive.

By means of the agglutinin fixation test, 19 specimens (63.3 per cent) were correctly grouped. Seven (23.4 per cent) did not give conclusive results. Four (13.3 per cent) were misdiagnosed because of 4 false negatives and 1 false positive reaction. The correct grouping of each of these 4 was made by the test for agglutinin content.

By evaluation of the findings in both tests on the bloodstains, with preference given to positive evidence, the final diagnosis was correct in 24 cases (80 per cent), while in 6 cases (20 per cent), the evidence was inconclusive; none were misdiagnosed. Specimen No. 11 was retested by the agglutinin fixation test and identified as Group A.

Of the 30 saliva specimens, 26 (86.7 per cent) were correctly grouped by the agglutinin fixation test. One specimen (3.3 per cent) was not definitely diagnosed. Three (10 per cent) were missed because of false negative reactions.

To determine the sensitivity of this technic of agglutinin fixation in terms of milligrams of dried blood, ten ⅛ discs of bloodstained cloth from various of the specimens used above were weighed; the weight of ten identical portions of unstained cloth was subtracted; the difference divided by 10 gave 0.84 mg. of dried blood per ⅛ disc or per 0.2 sq. cm.

A set of known stains showed no decrease in the activity of agglutinogens when last tested by this method at the end of five months.

Through the kindness of Dr. Karl Landsteiner in providing samples of anti-M and anti-N testing fluid, it has been possible to select individuals of types MN, M, and N. From preliminary tests with prepared stains, it appears that this technic is also adaptable to the demonstration of M factor.

DISCUSSION

Discussion of Technic.—In testing for agglutinins the lecithin suspensions recommended by Lattes have caused a slight desensitization of isoagglutination without better control of pseudoagglutination than is afforded by the use of 0 cells. Pseudoagglutination is not a problem in the agglutinin fixation test because of the dilution of the serum.

Lattes recommended the use of absorbing serum in a dilution equivalent to one-sixteenth of its end-titer. I believe that by exposing the stained material to an appropriate dose of undiluted serum a more intimate contact between agglutinins and agglutinogens and consequently a more thorough and rapid fixation is secured. The subsequent dilution to one-sixteenth of the definitive titer procures not only a working volume but also the optimum sensitivity for avoiding both nonspecific fixation and the masking of slight specific fixation which Lattes feared.

The centrifuge test method for demonstrating hemagglutination has been derived from Wiener and Vaisberg[13] to whom it was suggested by Landsteiner and Levine. Similar methods had previously been described by Levine and Mabee[14] and by Schiff.[15] The quantitative relationship between the resistance of the clumps to shaking and the titer of the test fluid has not been emphasized previously.

The technic described above for demonstrating agglutinogens is characterized by certain desirable features, namely:

a. It requires only about one-tenth the amount of material needed for most previous technics of agglutinin fixation or absorption.

b. The handling of materials is minimum, thus reducing the probability of technical error.

c. The speed makes for greater facility and for less bacterial interference.

The question of just what reduction in titer is of diagnostic significance is best answered by personal experience with the test. I regard a difference of

two grades (as C:2+; T:tr) as definitely diagnostic. However, in unchanged fluid the agglutination is surprisingly constant and a one-grade decrease in titer should be regarded with suspicion and rechecked.

In the experimental work, cells from various individuals have been preserved in Rous-Turner solution and washed in saline before use. For actual case work the cells and serum should be chosen from individuals of known group whom experience has shown to be free from atypical agglutination characteristics, and who will be available later if needed. The cells should be fresh and washed once in saline. Lattes recommends that the serum be separated at 0° C. to free it from cold agglutinins. Schiff prefers a mixture of serums of groups A and B adjusted to an equal titer for both factors. No serious difficulty has been experienced here in securing satisfactory Group 0 serums which do not require adjustment.

Discussion of Results.—In considering the experimental results it should be remembered that this technic is primarily designed for the examination of minimal stains unsuitable for the less sensitive method; that the figures are based upon a single examination; and that the attempt to demonstrate agglutinogens might have been several times repeated, still using less material than the first method mentioned.

Particular attention is called to the diagnoses obtained by correlating the findings of agglutinin and agglutinogen demonstrations. The greater percentage of correct diagnoses and the absence of false diagnoses leave no doubt of the desirability of attempting both demonstrations.

The results indicate that this technic is particularly suitable for demonstrating agglutinogens in saliva dried upon paper. The false negatives in the above series may be due to nonsecretion types; this has not been investigated.

Previously the demonstration of agglutinogens has been a second choice to the demonstration of agglutinins because it required a greater amount of material. Various technics require from 10 to 50 mg. of dried blood which can be lifted as a crust from the substratum, or at least 2 sq. cm. of bloodstained cloth. The test for agglutinin content is reported to be sensitive to 0.1 mg. of dried blood (crust?).

In using bloodstained cloth and the technic described above, I find that the demonstration of agglutinins requires more material than does agglutinogen demonstration. Moreover, the reliability of the test for agglutinins is inversely proportional to the age of the bloodstain, while the test for agglutinogens is not so affected.

General Discussion.—In this investigation interest has been centered upon the demonstration of agglutinogens because: these factors are more resistent to deterioration from age and physical exposure; they are found in body cells and secretions where the agglutinin content is low and inconstant; and finally only by such an approach will it be possible to demonstrate the factors M and N of Landsteiner and Levine.

The greatest objection to this test is that, since such a great part of our population belongs to Group 0, it will show only negative evidence in nearly half of the stains examined. Some workers have stated that a diagnosis of Group 0 should never be made by agglutinin fixation unless either M or N

agglutinogen is demonstrated. This is desirable but not always practical. A positive anti-human precipitin test is prerequisite to the grouping tests. In the presence of a positive precipitin test, negative evidence for A and B factors should be evaluated in the light of a definite history of the age and physical exposure of the stain. In favorable instances it seems reasonable to diagnose Group O without demonstrating either M or N factors. Repeated tests should always be made if the material at hand permits.

In forensic work the method of attack must be suited to the individual case after consideration of the legal problem as well as the material available. The theoretical limitations of blood grouping in solving the legal problem and the technical limitations in solving the scientific problem make conservatism imperative. If this attitude is maintained by workers undertaking forensic investigations, there is every reason to believe that such investigations may be in the future an invaluable aid in the administration of justice.

SUMMARY

A method for demonstrating group-specific substances in dried blood, by extracting with dilute saline, reconcentrating the extract and testing for agglutinins and agglutinogens after the manner of various European workers, gave satisfactory results in the hands of the investigator only when a sufficient quantity of material was available. Group identification of minimal amounts of bloodstained material by this method was time-consuming and uncertain.

A technic of agglutinin fixation sensitive to 0.84 mg. of dried blood is described. The test is made with a small amount of Group O serum allowing a forty-minute period for fixation. The serum is then diluted to one-sixteenth of its definitive titer and tests for fixation of the agglutinins are made by using A and B cells. For these tests the centrifuge test method is used in such a way that partial fixation of the agglutinins may be demonstrated and quantitatively estimated. The time required for this determination is about one hour as contrasted to twenty-four to forty-eight hours in the previous technic.

A technic for the demonstration of agglutinins in dried blood is described.

The results of examination by these methods of unknown blood and saliva stains from thirty individuals are presented.

While deterioration with age and exposure presents some difficulties in demonstrating agglutinins the desirability of attempting to do so is pointed out.

Appreciation is expressed for the invaluable suggestions and criticism of Dr. C. G. Culbertson; for the advice and constant encouragement of Dr. R. N. Harger; for the assistance of Dr. Phillip Kurtz in translation of the German references; and for the cooperation of fellow-workers in the preparation of unknown specimens and in the direct group determinations.

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A NEW RAPID METHOD FOR FROZEN SECTION DIAGNOSIS*

ARTHUR A. HUMPHREY, M.D., BATTLE CREEK, MICH.

THE method employed in this laboratory and in most institutions for the quick diagnosis of tissue biopsies has been that which has been described and modified by Broders, Terry, and others at various intervals during the last decade. This technic consists of floating the frozen section out on a solution of Terry's polychrome methylene blue, washing in water, and then mounting the stained section on a slide by means of a glass rod and dropping on a cover slip. The microscopic picture then presented shows excellent cellular detail and some color differentiation of the various types of tissue elements, and in the hands of an experienced tissue technician the results are uniformly good. One difficulty that is occasionally encountered is the presence of fat in certain blocks, such as in a breast tumor or axillary glands, where the fat globules obscure the picture and apparently alter its staining properties. Further difficulty is experienced in smaller institutions where the technician cannot devote sufficient time to sectioning alone to become technically proficient and biopsies may be so infrequent as to afford but little practice.

A method devised in this laboratory which has been used in conjunction with the above polychrome methylene blue has now almost replaced the latter as it is more rapid, simpler, and apparently "fool-proof."

This method in brief is as follows: A section is cut from the block on the freezing microtome in the usual manner and floated from a dish of water to a slide; the excess of water is wiped from the slide with a soft towel, care being taken not to touch the section. A drop of 1-200 (0.5 per cent) of brilliant

*From the Pathological Laboratory of the Leila Y. Post Montgomery Hospital.
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cresyl blue (Coleman and Bell) in normal saline solution is then dropped on the center of the section which stains it instantly, a cover slip is then placed on the stained tissue and the slide given a gentle tap downward on the finger to fix the cover slip in position, and the tissue is ready to be examined.

It will be noted that the dye covers the entire slide with the exception of the tissue, and it possesses a different color, further there is a pale or colorless halo immediately surrounding the section; this phenomenon obviates the possibility of a dark background which one might expect and renders washing after staining a superfluous step.

Although the polychrome method is sufficiently rapid for all ordinary surgical procedures the method just described is even more so, the reason being that staining is instantaneous and that the time required for washing is eliminated. A very definite advantage is the sureness with which good results are obtained, it being virtually impossible to over- or understain a tissue, and there is almost complete freedom from fat globules and air bubbles. The prepared sections for some obscure reason appear somewhat more resistant to drying out. The color differentiation is very similar to that of Terry's polychrome methylene blue, the fibrous or connective tissue assuming a reddish or purple tint, while the parenchymatous cells, mucus, etc., take varying shades from blue to green. The intracellular structure, however, appears somewhat different with the two dyes, the entire cell structure staining better with the Terry method, while the nuclear components appear more distinct with the vital stain.

I have often had occasion to cut sections on formalin fixed tissue from rapid diagnosis and have found this method very satisfactory; if polychrome stain were used on this tissue it would tend to overstain. In some types of unfixed tissue we have used 1 per cent solutions of brilliant cresyl blue, but in our experimental work solutions more dilute than 1-200 (0.5 per cent) are unsatisfactory. The solution does not seem to deteriorate and I have found that a fine tipped medicine dropper placed in the stopper of the bottle is a convenient way of dispensing it.

SUMMARY

A method is presented for staining in rapid tissue diagnosis which employs a vital stain—brilliant cresyl blue; it is simpler, more rapid, and more satisfactory in inexperienced hands than some methods in vogue.

A TECHNIC FOR PERFORMING A VALVULAR CECOSTOMY IN THE DOG*

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THE value of animals possessing a fistula in the large intestine at the level of the cecum is at once apparent to the investigator whose chief interest lies in the physiology and bacteriology of the colon. This type of opening has been made in dogs before, but leakage of intestinal contents has given difficulty. Animals with leaking fistulas are not easily kept in a good state of nutrition because of loss of water and salts, and also because of erosion of the body wall due to more or less continual contact with the intestinal discharge. This paper describes a cecostomy technic which includes the making of a valve which either eliminates leakage entirely, or reduces it to a point which permits a long normal life and obviates the necessity of continual bathing, bandaging, and nursing care.

PROCEDURE

Female dogs weighing from 12 to 15 kilos are treated with a vermifuge about one week previous to the operation. Preoperative treatment further consists in withholding food for forty-eight hours and allowing water *ad libitum*. We prefer to use morphine and ether as the anesthetics in our operations.

Although the cecum of the dog is usually located slightly to the right of the midline, just below the costal margin, it has been our practice to perform the operation on the animal's left side. When the necessity arises, as for instance in cases where a subsequent splenectomy must be performed, the right side is obviously the site of choice. We also make the incision in the abdominal wall as small as possible, contrary to the generally accepted surgical principle of making the incision large enough to permit all necessary manipulations with ease. In our experience the smaller incision has been an advantage, for when the intestine is placed in the body wall incision, there is considerable strain on the sutures closing the remainder of the opening and, as a result, the wound fails to close promptly or may even break open again; use of many stay sutures will not always prevent this.

First Stage.—An incision about 4 cm. long is made on the left side at the level of the last nipple lateral to the border of the left rectus muscle and about 5 or 6 cm. below the left costal margin. After cutting the skin, each layer of muscle and fascia is separated by blunt dissection. The peritoneum is incised and the edges are caught with several clamps. The operator's index finger is then inserted into the abdomen and an attempt made to locate the cecum. This frequently proves to be very difficult because of the small opening; often long sections of small intestine must be drawn out before the cecum can be located. As soon as the cecum has been brought into the operative field, it is kept care-

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fully bathed in warm physiologic saline solution. If this is not done and the organ is allowed to become cool, it rapidly contracts to about one-fourth of its normal size and presents great difficulty in the suturing process during the later stages of the operation.

The cecum is then dissected away with scissors from its mesenteric attachments to the small intestine. When the cecum has been completely separated from all of its attachments, it is placed in the incision and allowed to assume as nearly natural a position as possible. This position is not always the same. Sometimes the tip of the cecum shows a tendency to point cephalad, and at other times caudad. The direction in which it points aids materially in deciding the best position in which to place the cecum in the incision for suturing.

If it points cephalad, then the suturing should begin with the cecum placed in the caudal end of the incision; if the more natural position makes the organ point caudad, it should be sutured into the cephalad end of the wound. The advantage of this will be apparent when the sutures and procedures described below are considered. This point will be referred to later.

The cecum is drawn into the operative wound until it projects about 4 cm. through the body wall. Four or 5 sutures of No. 00 chromic catgut are taken in the base of the cecum on the medial side. These sutures penetrate the peritoneum, muscle and fascial sheaths of the body wall and the muscle layer of the cecum. When these sutures have been placed, the cecum is grasped by its tip and rotated so as to point in the opposite direction. This procedure produces not only a twist in the cecum but also a fold where the organ is bent over on itself; the production of the valve depends upon the successful maintenance of this twist and fold by suitable sutures. The cecum is held in position by the assistant while the operator places 4 or 5 sutures through the peritoneum, muscle and fascia of the body wall and the muscular layers on the lateral side of the cecum. Sutures are then placed along the base of the cecum and wherever may seem necessary until the peritoneal incision is entirely closed, and the part of the original incision not occupied by the cecum is properly closed. The skin is separated from underlying fascia for some distance at the point where the tip of the cecum lies; a suture of No. 00 chromic is then passed through the tip and then fastened to the abdominal muscle and fascia in the tunnel under the skin at the greatest distance possible without unduly stretching the cecum or injuring it in any way. This suture is designed to anchor the tip of the cecum in the cavity under the skin. The remaining edges of the skin incision are then fastened to the top of the cecum with No. 00 chromic catgut, care being taken to see that no sutures are so placed as to be subject to strain which will result in tearing the cecal issue. This terminates the first stage of the operation.

In a preceding paragraph reference was made to the advantage of placing the cecum in either the caudal or cephalad end of the body wall incision, depending upon what seems to be the natural position for the organ to assume. It should now be evident that, after having first anchored the cecum by stitches placed medially, then rotating the organ and maintaining it in that position by sutures properly placed, a portion of the cecum will extend beyond the end of the main incision or not, depending on whether the base of the cecum is sutured to tissues at that end or not. By so arranging matters as to make the exposed

cecum project beyond the end of the main incision and under the skin for some distance, one insures that the part of the cecum between the final fistulous opening (made in the second stage operation) and the valvular fold is provided with a firm membranous cover, namely, the skin.

The animals are not given any food on the first day after recovery from the anesthesia, and very little water is allowed. On the second day feedings of milk are begun, and on about the seventh day a stock ration. The dressings are changed every other day until the wound is completely healed.

Second Stage.—The second stage of the operation is carried out in from three to four weeks after completion of the first. At this time the area of skin where the cecum can be palpated as a long round mass is anesthetized with novocaine solution. At a point nearest the tip and farthest away from the bend in the cecum, a very small puncture wound is made with the tip of a scalpel. A metal cannula is then inserted, and an attempt is made to determine whether the opening is in the cecum. The test of this consists of palpation of the cannula within the intestine. If the test is negative, the wound is enlarged and the test repeated until there can be no question that the cecum has been incised. The edges of the cut cecum usually evert through the wound in the skin and are left in this position. We have observed no tendency for this opening to close, in contrast to our experience with gastric fistulas of the valve type. The animals are allowed to recuperate for about a week before being used for experimental purposes.

COMMENTS

We now have 4 dogs with valvular fistulas in the cecum made in this manner. All of our operations were performed over twenty months ago, but the animals are still in good condition. No difficulty has been encountered in maintaining their body weight, nor has there been excess leakage from the cecostomy openings. Two of the dogs show no leakage whatever, while the other 2 pass from 1 to 2 c.c. of intestinal material per day. Erosion of the body wall has not taken place in any of these animals. Experiments involving the insertion of a balloon into the large intestine by way of these fistulas have been carried out without difficulty. Materials of various kinds, including bacterial cultures, have been instilled without any harm to the animals. It is possible to flush out the cecum with any desirable liquid without difficulty. The little finger can be inserted without causing any untoward effects. The presence of the valve can readily be detected by the palpating finger.

CONCLUSIONS

A method of preparing a cecostomy with a valve in dogs has been described. Observations over a long period of time show that this type of operation can be carried out very easily and is in every way compatible with maintenance of an excellent state of nutrition and a long normal life in the operated animals.

A SIMPLE AGITATING DEVICE*

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A DEVICE capable of simultaneously stirring the contents of a number of beakers, or agitating small flasks, may be readily and simply constructed from wood or metal strips, using an ordinary automobile windshield wiper as the source of power.

We have had such a device in use for over a year constructed of thin oak slats 1 cm. wide, with accommodations for four flasks or beakers. The size of

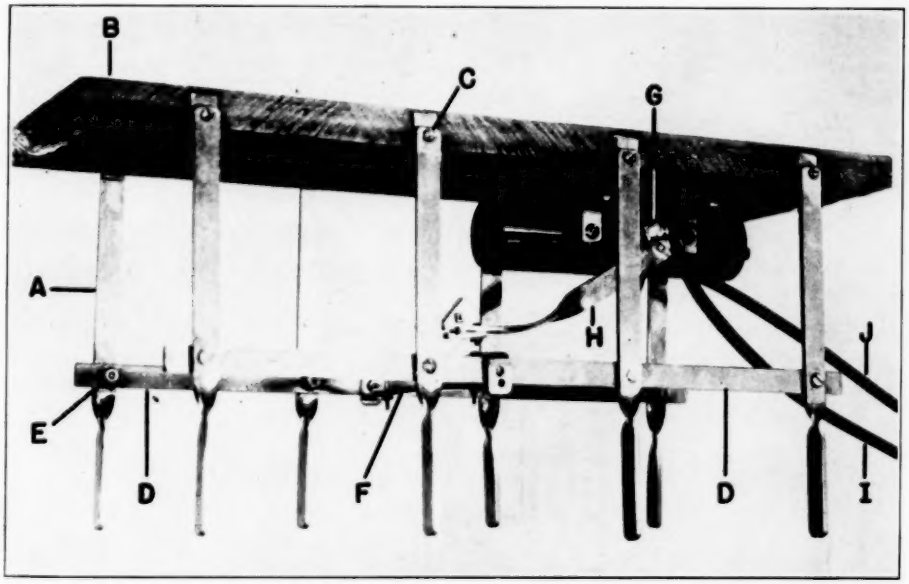

Fig. 1.—Photograph of an agitating device operated by a windshield wiper.

the apparatus may be varied to suit the purpose at hand. The one that we are now using is primarily for the purpose of stirring the contents of eight 600 ml. beakers simultaneously. It is shown in Fig. 1. Eight brass strips (*A*) 20 cm. long by 1.2 cm. wide, twisted at right angles in the lower third, are suspended from a rectangular board (*B*) by means of suitable bearings (*C*) so as to be free to move from side to side. The strips are spaced 10 cm. apart. A brass connecting rod (*D*) joins each set of four vertical strips with freely working bearings (*E*). The two connecting rods are joined by a rigid transverse bar (*F*). The reciprocating action of a windshield wiper arm (*G*) is communicated to the

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transverse bar by a connecting rod (*H*) with a bearing at each end. The excursions of the vertical rods (*A*) are controlled by the length of the windshield wiper arm, which may be made adjustable. Wipers may be obtained for operation on air pressure, vacuum, or by electricity.

Since our present set-up is maintained at body temperature, the board (*B*) is cut to such a length that it rests on shelf supports in a 37.5° C. incubator where it is securely anchored with a screw clamp. The wiper operates on the building air pressure line. The speed of the oscillating paddles is controlled by the quantity of air admitted through a needle valve. The valve is connected with the wiper by means of a rubber tube (*I*) leading into the incubator through a small opening, plugged with a two-holed rubber stopper. The exhaust air escapes from the wiper through tube (*J*) and out of the incubator through the second hole in the rubber stopper. This prevents the escape of cool air in the incubator which would cause fluctuations in the temperature. When the apparatus is in use outside of the incubator, it is clamped between two iron stands.

For mixing the contents of beakers, strips of glass, celluloid, or more recently, flat wooden "tongue-blades" are clamped to the free ends of the vertical oscillating rods (*A*) by the spring type of wooden clothespins. Small flasks may be agitated by fastening them to the oscillating rods by means of rubber bands.

The above described device has many adaptations in the laboratory, is inexpensive, simple of construction, practically noiseless in operation, and permits a reciprocating motion of easily controllable amplitude and rate.

A SECTIONAL TEST TUBE RACK*

For Use in the Titration of Serologic and Other Biologic Reactions

S. S. Lichtman, M.D., New York, N. Y.

THE sectional test tube rack was devised primarily for convenience in reading hemolytic reactions in connection with bile salt determinations in body fluids by the author's procedure depending on bile salt hemolysis.[1] This type of rack permits direct visualization of each entire row of tubes. Individual tubes do not have to be removed for inspection. In timed reactions this proves advantageous. The apparatus may also be used for the performance of agglutination, precipitation, hemolysis, and the Wassermann reactions.

DESCRIPTION OF APPARATUS

The rack consists of a stand and individual sections. The dimensions of the stand, length and number of sections, and the number and size of the holes are determined by the use for which the apparatus is intended.

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The Stand.—(Fig. 1.) The stand consists of a base and uprights and is made of noncorrosive metal if immersion in water is intended. The upper edge of the uprights permits suspension of the trays and shaking of their contents without dislodgement.

The Sections.—Each section is as wide as the diameter of the test tubes to be used. This permits close apposition of an entire row of tubes to the frosted glass surface of an illuminating box for precise differentiation between partial and complete hemolysis if an artificial source of light is used. Holes to accommodate agglutination or hemolysis tubes are punched in metal strips as close as possible to each other to facilitate comparison of the contents of adjacent tubes. The margins of the metal strips, bent at right angles tangentially to the

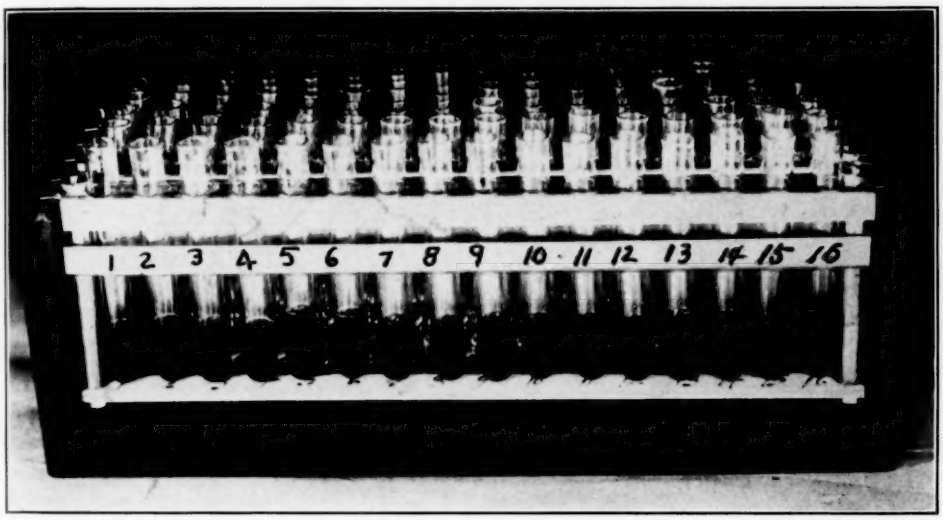

Fig. 1.—Rack with five sections.

holes, serve as flanges. A similar strip of metal with identical punching of holes is juxtaposed, their flanges almost meeting. A free view of the lower half of the tubes is thus afforded and the tubes are secure from falling out when their contents are agitated. The tubes rest on a platform. In agglutination or other procedures where the character of the sediment is examined through the bottom of the tube, holes may also be punched in the platform. Each section rests suspended on the uprights of the stand. The upper metal strip projects beyond the uprights of the stand to provide means for raising each section off the stand. Noncorrosive materials are used throughout.

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IMPROVEMENTS IN THE VARNEY ANAEROBIC JAR*

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THE Varney anaerobic jar[1] has been in daily use in this laboratory since 1927. In 1929, due to an unusual combination of circumstances, the author received severe burns in handling the phosphorus. However, the unique features of the jar prompted its continued use. Precautions were adopted to prevent further accidents. From time to time, modifications were adopted which resulted in distinct improvements. The present type of apparatus and the method of handling it will be described in this paper.

DESCRIPTION OF THE APPARATUS

The Glass Museum Jar.—The outer glass jar is a cylindrical museum jar similar to that originally used by Varney except that, because of the number of plates cultivated, a 5 by 18 inch jar is preferred. Such a jar will accommodate from 10 to 12 plates. These jars tend to crack near the flange, but this can be minimized by wrapping adhesive tape around the jar immediately beneath the flange. The assembled apparatus should never be carried by means of the metal clamp, as this imposes considerable strain on the flange. Water is poured in the jar to a depth of about one-half inch for absorption of phosphorus pentoxide fumes.

The Metal Rack.—The frame is made of Monel metal and heavily japanned. This minimizes corrosion inherent in earlier models. It fits inside the glass jar with sufficient play to allow for variations in the size of different jars. One of the upright strips of the frame is hinged at the lower end to permit filling the rack with Petri dishes. The feet extend 1½ inches below the base to keep the dishes out of the water. The top shelf of the rack is fastened to the frame and should be covered with bibulous paper. Metal straps extend 2¼ inches above the top and are used for lifting the rack in and out of the glass jar.

The Phosphorus Cup.—The space above the top platform of the metal rack accommodates the phosphorus container. By using a tall container for the phosphorus, reaching within one-half inch of the lid of the museum jar, there is little danger of the ignited phosphorus escaping into the jar. To keep the phosphorus as dry as possible, porous porcelain is used. Battery cups measuring 75 by 38 mm. sold by the Arthur H. Thomas Company as porous porcelain battery cups (No. 4378) have proved to be quite suitable. This cup can be manipulated with much less danger than the original Varney phosphorus assembly.

*From the Clinical Research Laboratory, 604 Fifth Avenue.
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METHOD OF IGNITING THE PHOSPHORUS

The most convenient form of phosphorus is that sold by the J. T. Baker Chemical Company under the label "Phosphorus, U. S. P., yellow, sticks, 3/16 inch" which is cut into convenient lengths. One of these pieces (about 1 inch long) is sufficient for a 5 by 18 inch jar.

The phosphorus is ignited by means of magnesium ribbon cut about 1½ inches long. It is best to wear "sun glasses." Ignite the ribbon in a Bunsen flame and put the incandescent magnesium into the cup containing phosphorus. The phosphorus will ignite immediately. Because of the violent reaction which follows, the lid of the jar must be put on immediately.

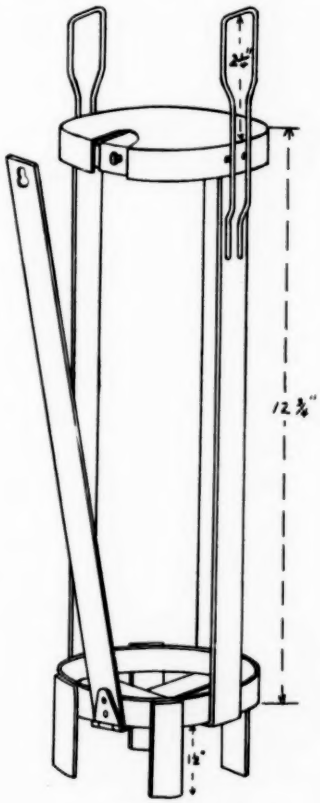

Fig. 1.

ASSEMBLING THE JAR

Loading.—The Petri dishes containing the cultures are interleaved with bibulous paper. They are stacked inside the metal rack which is then closed and lowered into the glass jar. Place the porous cup on the top shelf and, by means of long forceps, place a stick of phosphorus inside the porous cup. The phosphorus will not ignite for a while. Adjust the rubber gasket. Have the lid ready to set in place without delay. Ignite the magnesium ribbon and drop it into the porous cup. This ignites the phosphorus almost immediately. Cover quickly with the lid and clamp it tightly. Avoid drafts of cold air. Transport

the jar to the incubator by putting one hand under the bottom of the jar while steadying it with the other hand.

Unloading the Jar.—Have ready a 500 c.c. Griffin form beaker containing about 200 c.c. of water. Loosen the metal clamp from the jar and carefully pry off the glass lid. Immediately pour water into the porous cup. This prevents further ignition. This must be done quickly or the phosphorus will burn violently. Remove the battery cup by means of crucible tongs and immerse it in water. Very little residue is left in the cup after ignition because most of the combustion products are water soluble. The residual phosphorus in the bottom of the cup is left immersed in water ready for the next test. The porous cup is always kept under water until ready for use when it is drained off. The moist phosphorus is still capable of being ignited immediately by means of magnesium ribbon.

Several of these jars have been in daily use and have given complete satisfaction. They are admirable for anaerobic plating methods.

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A SIMPLE AND RAPID METHOD FOR OPENING SYRINGES CLOTTED WITH BLOOD*

George Holobaugh, New York, N. Y.

SYRINGES in which blood is allowed to clot often must be discarded because of the inability to remove the plunger. The following simple technic, which is an adoption of an old household principle, has been found very valuable in opening and cleaning "stuck" syringes.

Two pieces of soft rubber tubing, each 30 to 40 cm. in length (such as are used for tourniquets), are the only equipment required. The first tube is wrapped tightly around the barrel of the syringe in a counterclockwise direction so as to cover the whole barrel with one or more spirals of flattened tubing. This is then held tightly with the left hand while the second tube is wrapped tightly around the handle of the plunger in a clockwise direction, piling up to several thicknesses which can readily be grasped in the right hand. Force is then applied with the two hands to produce a clockwise rotation of the plunger within the barrel. Usually very little force is required but, if necessary, a powerful, steady torsion can be applied because of the improved grip and increased leverage. Careful and complete winding of both tubes will prevent injury to the operator if a breaking of the glass should occur.

This method has been employed successfully in opening syringes of all sizes in which dried blood has been allowed to stand even for many days.

*From the Medical Service, Presbyterian Hospital.
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A SIMPLE CATHETER DEVICE FOR THE CARE OF GASTRIC POUCH ANIMALS*

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EVERY investigator who has prepared gastric pouch dogs has been faced with the problem of controlling the erosion of tissue around the pouch opening caused by the digestive activity of the gastric juice. One of the usual methods employed in the postoperative care of such animals is the use of alkali to neutralize the acid of the pouch secretion, thus preventing peptic

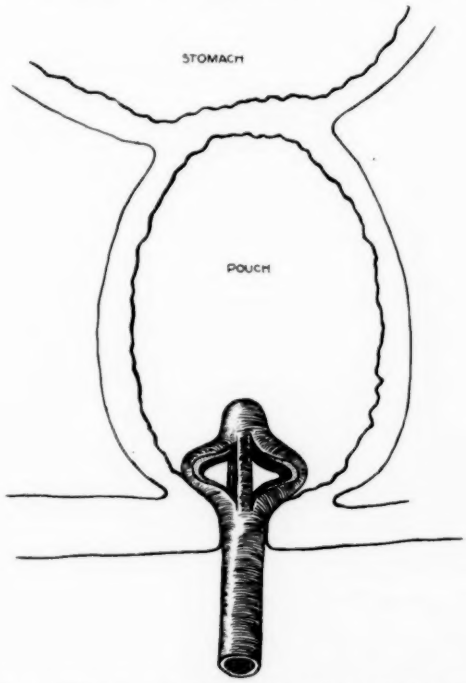

Fig. 1.—Schematic drawing of a self-retaining wing catheter in a gastric pouch.

digestion. Magnesium oxide, for example, is placed in gauze packs and kept in position over the fistula opening by means of abdominal binders of various types. Daily dressing is needed as a rule, and when the pouch is large or when the animal is fed a diet which stimulates excessive secretion, it is often necessary to change dressings twice a day in order adequately to control erosion. Despite the best of care, ulcerations do occur, and if the animal is by chance neglected for a few days, erosion through to the peritoneum is not unlikely.

It is obvious that such care is burdensome and time-consuming, especially so when one has several pouch animals requiring attention. We have been

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able to dispense with all dressings and alkali by the use of a permanently placed rubber catheter. The type used is either a wing or mushroom self-retaining catheter. It is placed in the pouch by means of a stylet. Fig. 1 illustrates the catheter in position in the pouch. The tube is first introduced after the dog has completely recovered from the effects of the operation. It is important that the tubes fit the pouch openings very snugly. For the larger openings a number 34 or 36 catheter has been found very satisfactory. About two inches of the tube stem is allowed to project beyond the body-wall. If the tube tends to be drawn into the pouch, one can prevent this by the use of a safety pin through the tube stem, or by placing a skin suture through the tube. Most animals ignore the presence of the tube, but an occasional one will develop the habit of pulling it out. This can quickly be discouraged by a skin suture which will keep the tube in place for days to weeks.

Fig. 2.—Photograph of gastric pouch opening with tube in place.

A very minimal amount of circumscribed erosion of the tissue immediately around the tube may occur due to peritubular leakage of secretion. This remains quite superficial and rarely extends more than a few millimeters beyond the mucosal junction. It does not progress and needs no special care. Occasionally a mild skin burn may occur on the hind legs due to the posture assumed in recumbency. The animals soon learn to avoid such occurrences.

An added convenience is the fact that this self-retaining catheter can be used for collection of samples. It is likely that the constant presence of these catheters may slightly increase the combined acid values. In two sacrificed dogs that had such catheters in situ for one and two months, respectively, an examination of the pouches revealed absolutely no noticeable mucosal changes.

To date we have employed the above device in five Pavlov and Heidenhain pouch dogs with complete success. It has also been used satisfactorily in an

antral pouch in a swine. One dog with a pouch secretion rate of 125 c.c. per hour at the peak in response to its daily meal has been maintained without dressings of any character entirely free of disturbing erosion for a period of over six months. In addition we have been able rapidly to heal extensive and deep ulcerations occurring in animals despite daily dressings with alkali. Fig. 2 illustrates the excellent condition of the tissues surrounding the self-retaining catheter in a Pavlov pouch dog.

The authors are indebted to Dr. Louie N. Claiborn of the Department of Surgery for the suggestion that such a device might prove of value.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

THYROID FUNCTION, Iodine Tolerance Test for the Investigation of, Watson, E. M., and Barber, A. S. Endocrinology **20:** 358, 1936.

An iodine tolerance test is described whereby the relative rate of disappearance from the blood stream of intravenously injected iodine may be expressed. The results of the application of the test in 30 cases are recorded.

In 13 of 16 individuals with clinically normal thyroid glands, from 9 to 23 per cent of the injected iodine remained in the blood stream 6 hours after its injection. In 6 of 10 patients with thyrotoxicosis not any of the injected iodine remained in the blood stream after six hours and in none of these cases was more than 5 per cent of the injected iodine found to be present at this time. In 4 cases of hypothyroidism the average quantity of iodine in the blood 6 hours after its injection was greater than normal.

These few observations suggest that the iodine tolerance test may be a means of providing evidence of diagnostic importance in cases of doubtful thyroid disease.

The technic employed for performing the iodine tolerance test is briefly as follows. With the patient in the fasting state in the morning, an amount of Lugol's solution containing 250γ of iodine per kilogram of body weight, after being diluted with 15 c.c. of 0.85 per cent NaCl solution, is injected intravenously. Samples of venous blood of about 12 c.c. each are obtained immediately before the injection and five minutes, two, four, and six hours afterward. These samples are received in tubes containing a small amount of potassium oxalate which serves as an anticoagulant. Food is withheld from the patient during the test period.

The concentration of iodine in each sample of whole blood is estimated by means of a method described by Perkin. In this procedure 10 c.c. of blood are placed in a nickle crucible together with 2 gm. of potassium carbonate and combusted on a hot plate and in a muffle furnace for four and one-half hours. The charred mass is extracted with alcohol, filtered and the filtrate is evaporated to dryness. The residue which remains is dissolved in water and when the solution is made slightly acid with H_2SO_4 and a drop of freshly prepared bromine solution is added, the iodine is oxidized to iodate. The addition of potassium iodide frees the iodine which is estimated by titration with 0.001 N sodium thiosulphate solution with starch serving as an indicator.

The iodine content of the blood specimen secured five minutes after the injection of the Lugol's solution minus that of the preliminary control sample, is regarded as representing the maximum increment caused by the injected iodine and is consequently recorded as 100 per cent. With this value as a basis, the findings for the other samples are expressed accordingly. While the results so obtained represent the relative rather than the absolute iodine concentrations, they do provide an indication of the rate of disappearance from the circulating blood of the injected iodine in a specified time.

KIDNEY, Hypernephroid Carcinoma of, With Tumor-like Thrombus Filling the Inferior Vena Cava and Right Heart Cavities, Woodruff, L. W., and Levine, V. J. A. M. A. **106:** 1544, 1936.

A case of tumor-thrombus of the inferior vena cava, arising from a hypernephroid carcinoma of the kidney and extending into the right atrium and ventricle, was not diagnosed during life, largely because of the absence of edema of the lower extremities. Circulation time tests might make a correct diagnosis possible in this type of case.

PNEUMOCOCCIC INFECTIONS, Type Specificity in Pneumonia and, Kohl, C., and Reitzel, R. J. J. A. M. A. **106:** 1557, 1936.

In an interval of three years, from December, 1932, to December, 1935, pneumococci were found in 353 patients, of whom 322 adults (91 per cent) had pneumonia.

During the last twenty-three months of this study, diagnostic serums of all available types, that is, I to XXXII, were used. Eighteen types other than the original three were found in either pneumonia or other infections. Only two strains were found that failed to be classified in Types I to XXXII.

In pneumonia, Type I was the most frequently encountered type, having been found in 138 (42.8 per cent). The order of prevalence of the other types was: Type II, 12.1 per cent; Type III, 8.5 per cent; Type VII, 4.3 per cent; Type VIII, 2.4 per cent.

The incidence, bacteremic rates and mortality of Type I (42.8, 49.2 and 44.1 per cent respectively) has been found to be higher than elsewhere.

Seventeen types other than the original three were found in fifty cases of pneumonia. From this small series, conclusions cannot be drawn as to the individual clinical characteristics of these types in this locality.

The Neufeld method for typing has been found to be satisfactory.

ALCOHOL ADDICT, Observations on the Etiologic Relationship of Vitamin B to Polyneuritis in the, Jolliffe, N., Colbert, C. N., and Joffe, P. M. Am. J. M. Sc. **191:** 515, 1936.

The vitamin B and caloric intake of 42 alcohol addicts, 26 of whom had polyneuritis, have been estimated quantitatively by Cowgill's formula. When the first 1,600 calories contained in the alcohol consumed by each subject are included in the estimation of the vit/cal ratio, a definite correlation is found between the inadequacy or adequacy of the vitamin B intake and the presence or absence of polyneuritis. This correlation is as follows:

1. Every alcohol addict with polyneuritis had an estimated inadequate vitamin B intake.
2. No alcohol addict with an estimated adequate vitamin B intake had polyneuritis.
3. Every alcohol addict with estimated absolute deficiency of vitamin B for twenty-one days or more had polyneuritis.
4. Polyneuritis may develop in an alcohol addict as early as the 7th day of estimated absolute deficiency of vitamin B.

The authors' data substantiate the hypothesis that polyneuritis in the alcohol addict is due to the lack of vitamin B by demonstrating: (1) that the diet consumed by alcohol addicts with polyneuritis failed quantitatively and over a sufficient period of time to contain adequate amounts of vitamin B as compared with the predicted requirement; and (2) that the diet consumed by alcohol addicts without polyneuritis, though the addiction was of long duration, contained quantitatively adequate amounts of vitamin B as compared with the predicted requirement.

It is therefore concluded that alcohol has no direct toxic action (chronic) on the peripheral nerves, and that polyneuritis in the alcohol addict is due to vitamin B deficiency.

HODGKIN'S DISEASE, The Gordon Test for, Rosenberg, D. H., and Bloch, L. J. A. M. A. **106:** 1156, 1936.

Method.—With aseptic precautions a lymph node is collected in a sterile test tube or Petri dish. One or more grams is placed in a small sterile mortar under cover, and the rest of the node is kept in a refrigerator. This tissue is cut into very small pieces and is ground into a fine emulsion, nutrient broth of pH 7.1-7.2 being added to make a 1:10 suspension. It is maintained in a refrigerator at from 0 to −4° C. for seven to ten days, and, before using, is tested for sterility aerobically and anaerobically. All lymph nodes in this study were obtained from live patients. If necropsy material is studied, the node should be removed aseptically, disinfected with absolute alcohol, and dipped into boiling water and then into ether. Phenol (0.5 per cent) may be added to the broth as a further precaution against contamination.

In rabbits lightly anesthetized with ether, a short incision is made in the scalp and a tiny opening is drilled in the skull at a point 2 mm. lateral to the sagittal suture and 1.5 mm. anterior to the lambdoidal suture. Through this opening, 0.4 c.c. of sterile suspension is slowly injected into the occipital lobe to a depth of about 3 mm. by means of a short fine needle. The skin is then sutured and the wound covered with collodion. In addition, 0.6 c.c. of the inoculum is injected into the marginal ear vein. The rabbits are weighed before the inoculation and daily thereafter.

Positive Test.—After an incubation period of from two to six days (in one rabbit, sixteen days), slight impairment of locomotion sets in, followed by a progressive spastic paralysis of the hind limbs. The gait becomes decidedly ataxic, and the animal may stagger and fall. In the more marked reactions, retraction of the head, opisthotonos and convulsive movements are observed. Van Rooyen reported nystagmus, grinding of the teeth and urinary and fecal incontinence in some animals. Progressive wasting and weakness, affecting particularly the hind quarters, are found in all cases. Death occurs frequently within three days to one month, usually in ten days. A larger number recover completely or pass into a chronic state.

At autopsy, gross examination reveals only a slight hyperemia of the meninges. Microscopically, usually little or no cellular infiltration in the brain is found, but with more active material Gordon noted a lymphocytic meningitis and perivascular lymphocystic infiltration of the brain substance. No inclusion bodies have been described. Cultures of the brain and meninges are sterile.

DIARRHOEA, Infectious, An Epidemic of, in the New-Born, Barenberg, L. H., Levy, W., and Grand, M. J. H. J. A. M. A. **106**: 1256, 1936.

An epidemic of infectious diarrhea, parenteral in origin, occurred, thirty-two infants presenting a characteristic syndrome.

There was no relationship between the normal type of feeding and the incidence of the disease.

Dehydration was evident, but true alimentary intoxication was noted in only one instance.

Otitis media and bronchopneumonia as complications were frequent.

The severity of the disease decreased with the return of alkaline stools.

Bacteriologic studies failed to reveal a causative organism.

The administration of 1 per cent salt solution orally was well tolerated and obviated the use of the intravenous route for nutrition or medication.

Necropsies did not show a pathogenic picture in the gastrointestinal tract to indicate a primary intestinal infection.

The finding of pneumonia with mononuclear infiltration in the absence of any intestinal lesions suggested the possibility of a virus infection.

RENAL CARCINOMA, Prognosis in, Braasch, W. F., and Griffin, M. J. A. M. A. **106**: 1343, 1936.

As an index to prognosis and in the interest of simplified terminology it would seem best to regard all malignant tumors of the renal cortex as carcinomas and to grade them according to the degree of cellular differentiation.

From a clinical point of view, carcinoma of the renal cortex may be divided further into two groups which have distinct clinical characteristics; namely, adenocarcinoma (hypernephroma) and alveolar carcinoma.

Although they may be various factors, such as metastasis, which can modify postoperative results, the prognosis usually will conform to the histologic evidence of malignancy.

Metastasis occurs most frequently with renal tumors of the higher grades of malignancy and may be present without causing clinical evidence. Lymphatic extension is a frequent occurrence. Metastasis is found most often in the lungs, rather infrequently in the osseous system. Although the progress of the disease in the presence of pulmonary

metastasis may be delayed by nephrectomy and irradiation of the lungs, postoperative results indicate that the chance for recovery is so slight that operation is not justified. It is possible that the roentgenographic evidence of pulmonary metastasis in some of the few cases in which patients were reported as having recovered was incorrectly interpreted.

Calcification of tissues in cases of renal adenocarcinoma is a frequent occurrence and indicates a favorable prognosis. Urographic evidence of widespread involvement of all calices and of the pelvis indicates a high grade of malignancy and a guarded prognosis. The excretory urogram will give accurate information with regard to the presence of renal neoplasm in a high percentage of cases. Its routine use should be more frequent in the identification of abdominal tumors.

Although clinical data suggest that suprarenal elements may be included in some hypernephromas to account for the vascular manifestations such as hypertension and telangiectasis, neither chemical analysis of the tumors nor postoperative clinical data corroborate such an assumption.

While the size of the tumor alone has no bearing on the late postoperative results, nevertheless a large, fixed tumor together with a short history and evidence of marked toxemia would indicate a bad prognosis and would accordingly render the advisability of operation questionable.

TRICHINOSIS, Laboratory Diagnosis in, Heathman, L. S. Am. J. Hyg. **23**: 397, 1936.

In the author's hands the intradermal skin test and the precipitin test appear to be of much less value in the laboratory diagnosis than do the eosinophile count, together with muscle biopsy, and the study of the meat suspected of being the source of infection.

Intradermal skin tests and precipitin tests in animals heavily infected with *Trichinella larvae* gave even a lower percentage of positive reactions than that found in human beings. Animals did not tend to develop positive intradermal reactions regularly after being tested a number of times. The intradermal tests in both man and animals are less clear-cut and more difficult to read than a number of other diagnostic intradermal tests.

It would seem very important both from the theoretical and practical standpoint that skin tests in trichinosis be more thoroughly studied.

PSITTACOSIS, Laboratory Investigation of, Burnet, F. M. Med. J. Australia **1**: 363, 1936.

If the suspected bird is available it should be handled with great care to avoid inhalation of dust from the feathers and feces, and preferably drowned immediately in a solution of some weak antiseptic. The body cavity is opened with aseptic precautions and note made of any lesions present. In acute psittacosis of cockatoos, subacute pericarditis and inflammation of air-sac linings are almost invariable. From the semipurulent lymph, smears should be made and stained by Castaneda's method. The liver is usually enlarged and flabby, with usually some variation from the normal color: it may be brownish green or, more rarely, may have yellowish necrotic patches. The spleen is usually moderately enlarged, and should be removed for inoculation into mice. For this purpose it is ground with a little sterile quartz powder or sand and two cubic centimeters of broth or saline solution. After the abrasive has been allowed to settle, quantities of 0.5 c.c. are inoculated intraperitoneally into mice.

The mice should be kept under conditions which will minimize the possibility of infecting the workers in the laboratory. Individual glass jam jars with perforated screw tops for each mouse are satisfactory. If pneumococci are present in the suptum, the mouse will die within three days and may be discarded. If it survives any bacterial infection, psittacosis may be manifested by signs of sickness in the mouse from four days onward. It should be killed as soon as it is obviously sick, and the abdominal cavity examined. Subacute peritonitic lesions with fibrinopurulent accumulations in the subphrenic regions and around the spleen are characteristic of Australian strains of psit-

tacosis. Only very rarely are necrotic patches on the liver to be seen. The spleen is enlarged to a variable extent. Smears should be made from the inflammatory exudate and stained by Castaneda's method. The stain is prepared as follows:

Phosphate buffer solution (pH – 7.0)	95 c. cm.
Formalin	5 c. cm.
Loffler's methylene blue	10 c. cm.

Smears are stained with this for two minutes, without preliminary fixation, washed with distilled water, and then counterstained with 1 per cent aqueous safranin. Examined under the oil immersion lens, the specific psittacosis bodies (L.C.L. bodies) appear as blue granules, about 3 microns in diameter, mostly in small clumps within mononuclear cells. In a properly stained smear all other components stain pink. Bacteria also stain blue in many instances, but are easily recognized by their size and shape.

If mice do not appear sick by the tenth day, it is advisable to kill and examine them. If nothing abnormal is visible and the spleen is not enlarged, they may be considered uninfected. If the spleen is enlarged and there is a suspicion of peritoneal exudate on some of the peritoneal folds around the lesser omentum, but no L.C.L. bodies in smears, it is advisable to emulsify the spleen and subinoculate it into two fresh mice. Cultural tests of the spleen should be made at the same time. If the virus is present, this second mouse passage will give typical findings.

The virus is readily inactivated, and if material has to be sent some distance to the laboratory it is advisable to pack it with ice, preferably in a thermos flask, during transit.

LIVER, Clinical Value of Test for Hippuric Acid in Disease of the, Quick, A. J. Arch. Int. Med. **57:** 544, 1936.

The excretion of hippuric acid after the administration of sodium benzoate was studied in a series of one hundred cases, in fifty-eight of which hepatic or biliary disease was present. A patient with a normally functioning liver excretes approximately 3 gm. of benzoic acid in the form of hippuric acid in four hours after taking 6 gm. of sodium benzoate. A low output of hippuric acid occurs in cases of catarrhal jaundice and various forms of hepatitis and usually in cases of a malignant process with metastasis to the liver, syphilitic cirrhosis and atrophic and hypertrophic cirrhosis. Reaction to the test is normal in cases of cholecystitis, cholelithiasis and biliary obstruction due to stones in the common duct if the condition is of short duration. The test appears as a promising means for estimating hepatic insufficiency and as an aid in the differential diagnosis, especially in distinguishing between the jaundice of hepatitis and the jaundice arising from obstruction due to a stone in the common duct. The test is useful in following the course of disease of the liver and in determining the effectiveness of therapy.

TONSIL, Malignant Lymphoma of, Jackson, H., Jr., Parker, F., Jr., and Brues, A. M. Am. J. M. Sc. **191:** 2, 1936.

In fifteen years there have been admitted to the Huntington Hospital 236 cases of malignant disease of the tonsil. Of these, 28 (11.8 per cent) were proved to be malignant lymphoma of one type or another.

The most prominent symptoms initially noted by the patients were persistent sore throat, swelling within the throat and enlargement of the cervical lymph nodes.

Of these patients 56 per cent developed generalized lymphoma.

The excellence or rapidity of the immediate response to radiation is no index of the ultimate outcome.

The average duration of life from the onset to death was 2.6 years, the median 1.4 years, and the extremes were 3 months and 13 years.

One patient was alive 5 years, 2, 10 years and 1 alive and free from symptoms 18 years after the onset.

The absence of local lymph nodes at the time of treatment is, in general, of good prognostic import.

Lymphoma of the tonsil should be regarded as but one type of malignant lymphoma and the ultimate widespread involvement seen in many patients must constantly be borne in mind.

It is suggested that those patients be treated as if they had carcinoma of the tonsil; that is, with very heavy initial radiation with comparable doses for recurrences.

MUCOSA, Are There Cyclic Changes in the Human Vaginal? Zondek, B., and Friedmann, M. J. A. M. A. **106:** 1051, 1936.

In the human vaginal mucosa the authors could not find cyclic changes analogous to those of the uterine mucosa.

The vaginal mucosa shows different microscopic pictures in different places.

In deficient ovarian function (primary amenorrhea) is found a picture of a mucosa similar to one with good ovarian function, with even the same changes as in the premenstrual phase.

In the absence of ovarian function, by means of ovarian hormones (estrogenic substance, progestin) the authors could produce enlargement of the uterus, a proliferative and premenstrual uterine mucosa and menstruation, but could not find analogous changes in the vaginal mucosa.

In human beings the infantile vagina may be influenced by estrogenic substances, but it is not certain whether it is because of a specific hormone effect on the vaginal mucosa or on the mucous membranes in general.

Since the vagina is developed embryologically different in different species, the different reaction of the vaginal mucosa is explainable.

RETICULOCYTES, The Resistance of, to Hypotonic Solutions of Sodium Chloride and of Plasma, Daland, G. A., and Zetzel, L. Am. J. M. Sc. **191:** 467, 1936.

The absolute number of reticulocytes observed in 100 cases of pernicious anemia, hypochromic anemia, and hereditary or acquired hemolytic jaundice showed no consistent effect in altering the minimum resistance or maximum resistance of the total red blood cell population to hypotonic sodium chloride solutions.

Actual enumeration of the relative numbers of reticulocytes and red cells surviving in progressive series of hypotonic solutions of sodium chloride and of plasma showed that, irrespective of the disease present, in some bloods the reticulocytes were more resistant, in some equally resistant, and in some less resistant than the red blood cell population as a whole in the cases studied.

Reticulocytes as well as nonreticulocytes vary in age, volume, thickness, hemoglobin content, and probably other physical and chemical properties in the same individual at different times, and in different individuals with varied disease conditions. Thus the fact that young cells are reticulocytes does not necessarily determine their behavior in hypotonic solutions. The reticulocytes may or may not exhibit divergence from the resistance of the cell population as a whole. Such variations may be responsible for the diverse opinions of other observers in regard to the resistance of reticulocytes to hypotonic solutions of sodium chloride and plasma.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Delafield and Prudden's Textbook of Pathology*

THIS is a book which needs no introduction, having been for fifty years one of the best-known and most popular of all reference texts in pathology.

It is the one text on this subject to which this reviewer has never turned without finding something pertinent to the problem at hand.

The present revision by Dr. Wood is, as he says, in one sense a jubilee publication, the first edition having appeared in 1885.

It will be found thoroughly revised, especially in the section on the Nervous System, and the present volume, as heretofore, remains one of the most useful of all reference texts on this subject.

As always, Delafield and Prudden can be recommended not only without reserve, but with enthusiasm.

The Bacteriology of Typhoid, Salmonella, Dysentery and Carrier States†

AS DR. HAVENS remarks, in the opening chapter of this book: "There is, perhaps, no more ubiquitous group than the Salmonella and dysenteries, nor one that offers, on the one hand, such technical pitfalls in its study and, on the other, so much satisfaction when these technical difficulties are crowned by successful isolation and identification."

Few there are who have ever been confronted with these problems who will hesitate to agree with this summary of the difficulties they present; and all within whose province such problems lie will welcome this small but excellent book devoted to methods for their solution.

In view of the unwarranted degree to which clinical reliance has been and still is placed upon agglutination reactions are accepted as diagnostic of these infections, the book begins with a clear description of the antigenic composition of the bacterial cell.

Then follows a discussion of bacterial variation, particularly as related to antigenic variations. There is an excellent discussion of the culture media best suited to the isolation of pathogens from fecal specimens; the procedures cultural and serological applicable to the identification of intestinal pathogens; after which the various organisms are discussed in detail. The carrier problem is discussed at length.

Bearing on every page inherent evidence of the extensive experience of the author, it is certain that this volume will become a most valuable reference text for the laboratory worker, the health department, and the clinical laboratory in general.

It may be recommended without reserve as a valuable and most useful contribution on a subject of general importance.

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*Delafield and Prudden's Textbook of Pathology. Revised by Francis Carter Wood, Director, Pathological Department of St. Luke's Hospital, New York, etc. Cloth, 1406 pages, 22 plates and 839 text illustrations. William Wood & Co., Baltimore, Md.

†The Bacteriology of Typhoid, Salmonella, Dysentery, and Carrier States. By Leon C. Havens, M.D., Director of Laboratories, Alabama State Department of Health. Cloth, 158 pages, 6 figures. The Commonwealth Fund, New York.

Blood Groups and Blood Transfusions*

AS DR. WIENER indicates in his preface, studies of the individual differences in human blood have been extensive and somewhat involved.

The practical application of the data evolved, not only to the transfusion of blood but, more recently, to various medicolegal aspects such as the identification of blood stains and the determination of paternity, have made it incumbent upon physician and laboratory worker alike to become familiar with the subject. To these this book will come as a welcome contribution.

The reputation and experience of the author in this field assures the reader of a thorough, balanced, and authoritative discussion, an expectation which will not be disappointed.

The subject matter is so arranged and discussed as to furnish all the particular information desired. The advanced worker in this field as well as those to whom it is unknown alike may read this book with profit.

This volume will deservedly take rank as a reference text par excellence in this field.

Stone in the Urinary Tract†

DESPITE the frequency and importance of calculus formation in the urinary tract, this subject is seldom adequately dealt with in the average textbook.

As a subject of practically monographic proportions it is, hence, best and most adequately discussed in a monograph devoted to it alone. Such an endeavor the author has carried out with no small measure of success.

This book is well conceived and, by virtue of the author's wide and comprehensive experience, well carried out. Beginning with the etiology, chemical composition, and pathology of calculus formation, the text considers in the order named the varied features of renal, vesical, prostatic and preputial calculi with particular attention in each instance to the clinical symptomatology, diagnosis and treatment. A separate chapter is devoted to urinary calculus in children and another to urinary calculus in relation to pregnancy.

The book is well and clearly written and, by the use of heavy type, significant portions of the text are brought into relief. The text is copiously and well illustrated. An extensive bibliography is attached covering all the references to urinary calculi from January, 1910 to June, 1928.

This volume should prove valuable to the urologist, the pathologist, and the physician at large as presenting in a clear and comprehensive manner the present information available on this subject. It can be thoroughly recommended.

The Relief of Pain‡

THIS is a book which should prove of inestimable value to the practitioner. For not only is pain the dominant symptom which drives the vast majority of patients to the physician but it is without doubt the one symptom for the relief of which they are most unanimously clamorous.

The hints, suggestions, and methods presented in this book should prove, therefore, most welcome to the physician.

It must not be inferred that Dr. Balme discusses pain solely as a symptom to be treated. On the contrary, while its relief is often indicated and of definite importance in the manage-

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*Blood Groups and Blood Transfusions. By A. S. Wiener, M.D., Cloth, 220 pp., 41 figures and 71 tables. Charles C. Thomas, Springfield, Ill.

†Stone in the Urinary Tract. By H. P. Winsbury-White, Assistant Urological Surgeon, St. Paul's Hospital for Genito-Urinary and Skin Diseases, etc., etc. Cloth, 344 pages, 2 colored plates and 181 text figures. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

‡The Relief of Pain. A Handbook of Modern Analgesia. By Harold Balme, M.D., Formerly Professor of Surgery and Dean of The School of Medicine, Cheeloo University, China. With an Introduction by Sir E. Farquar Buzzard, Regius Professor of Medicine, University of Oxford. Cloth, 392 pages. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

ment of the underlying condition responsible for its production, Dr. Balme wisely emphasizes that pain is, after all, a symptom and a sign and recalls the dictum of Hilton that "every pain has its distinct and pregnant signification if we will but carefully search for it."

As the author says, further: "The call of pain presents a double appeal to every conscientious physician. It is at once a call to investigation and a call to relief and neither can be truly obeyed without the fullest recognition of, and response to, the other."

The first part of the book discusses the problem of pain, its nature, pathway, its relation to physical phenomena, the sensitivity of the individual to pain, its classification, and methods for the investigation of pain.

Part II discusses general and systemic pain together with methods for its control, while Part III discusses regional pain and methods for its relief.

Part IV is devoted to the therapeutics of analgesia.

The book is well and clearly written and bears the hallmark of evaluated experience and critical analysis of the pertinent literature. There is a comprehensive index.

This book can be recommended as an eminently useful and practical text.

Parenteral Therapy*

THIS book is, apparently, an outgrowth of one first written by the senior author in 1924 under the title of "Intravenous Therapy."

As the title indicates, its purpose is to present all methods for the administration of medicaments except the alimentary route.

The first section (155 pages) presents in detail and with excellent and ample illustrations the general technic of parenteral therapy. This section should be of great practical value because of its clear and full descriptions of technic.

The second section is a therapeutic index in which a large number of conditions are listed alphabetically each followed by a list of drugs, etc., applicable to their treatment.

The third section under the heading pharmacologic notes presents an alphabetical list of drugs and various preparations which may be administered parenterally, giving their action, dose, and method of administration.

A list of their distributors is appended.

The book is well indexed and in a relatively small space embodies a large amount of information of practical value. It should be favorably received and should prove of great usefulness to all who may from time to time desire to administer medicaments parenterally and thus to physicians, dentists and veterinarians.

Erratum

In the article "Schilling's Hemogram" by Emil Maro Schleicher in the September, 1936, issue of the JOURNAL, the last sentence on page 1298 should read: "From the data obtained, the relative average ratio per low power field is determined by adding the columns and dividing the total numbers into each other."

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*Parenteral Therapy. By Walter Forest Dutton, M.D., Formerly Medical Director, Polyclinic and Medico-Chirurgical Hospitals, Graduate School of Medicine, University of Pennsylvania, etc., and George Burt Lake, M.D., Formerly Special Lecturer in Hygiene, Purdue University, etc. Cloth, 386 pages, 90 half-tones and engravings. Charles C. Thomas, Springfield, Ill.